KU LEUVEN

FACULTY OF PSYCHOLOGY AND EDUCATIONAL SCIENCES

RESEARCH GROUP ON HEALTH PSYCHOLOGY

Unpleasantness and Fear in INTEROCEPTION

Doctoral thesis submitted to obtain the degree of Doctor (PhD) in Psychology

by **Erik Ceunen**

Doctoral advisor: Prof. Dr. Ilse Van Diest

Co-advisor: Prof. Dr. Johan W.S. Vlaeyen

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"If there must be an absence of pain for joy to exist, then the individual is doomed. We must find a way to experience joy despite our pain, because everyone is fighting their own secret battle. Everybody hurts. Everybody."

- *Edward Yoo*

Erik Ceunen

Unpleasantness and Fear in Interoception

Dissertation submitted to obtain the degree of Doctor in Psychology (PhD), 2015
Advisor: Prof. Dr. Ilse Van Diest; co-advisor: Prof. Dr. Johan W.S. Vlaeyen

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Interoceptive fear (IF) is the apprehension of bodily sensations. IF is relevant to a number of psychiatric and somatoform disorders, in which fear learning to bodily sensations has been hypothesized to play an important role. Although IF conditioning (IFC) is of relevance to understanding the etiology of these disorders, thus far research has hardly addressed the basic fear response topography to experimentally induced interoceptive sensations.

Human fear can be measured through self-report inquiring about subjective fear, unpleasantness, arousal, sense of control (dominance), and the extent to which an aversive event is expected (expectancy). A validated psychophysiological measure for the motivational intensity, i.e. for arousal, is skin conductance. Eye blink startle is modulated by motivational direction (pleasant vs unpleasant) and is used to measure it. During unpleasantness and fear, startle magnitudes are generally potentiated compared to emotionally neutral and pleasant states.

The primary aim of this doctoral project was to find out whether the human eye blink startle paradigm can be used to measure defensive response mobilization elicited by aversive interoceptive stimulation. To this purpose, a two-step approach was used: in a first step the concept of interoception was critically reviewed, while in a second step a series of empirical studies were conducted.

The review on interoception traced the development of the concept to its modern day usage. This review proposes to consider interoception as the phenomenological perception of the state of the body, irrespective of how this perception is formed and whether it is accurate or not. Labels are suggested for specific components of interoception that allow to classify sensations as similar or distinct.

In the first study, participants adopted a flexed, an upright, and an extended posture. The reasoning was that postures can influence emotions, and thus perhaps impact startle. Also, differences in startle had previously been observed in gastrointestinal (commonly associated with a flexed posture) vs respiratory (extended posture) stimulation. Study 1 found startle magnitude to be higher during the extended posture, due to negative affectivity associated with that posture.

In a second study, healthy participants were exposed to a cold pressor test (CPT), loaded breathing, and inhalation of CO_2-enriched air. Startle amplitudes during interoceptive stimulation were decreased relative to when there was no stimulation. Respiratory stimuli showed a linearly decreasing slope during prolonged stimulation, while the CPT evoked a quadratic pattern.

In the third study we observed a potentiated startle during painful esophageal stimulation in women, but not in men. In the fourth study, we used esophageal stimulation to establish a homoreflexive interoceptive conditioning paradigm in which a non-painful esophageal stimulus (conditioned stimulus, CS) preceded a painful esophageal stimulus (unconditioned stimulus, US) in a paired, but not in an unpaired group. Compared to the latter, participants from the paired group learned to fear the CS, as reflected in their US-expectancy ratings and skin conductance response. Women in the paired group also showed a trend towards increased startle in response to the CS (relative to a safe period). This response disappeared again during the extinction phase; on the other hand, in the extinction phase men in the paired group showed higher startle amplitudes during the CS.

In summary, our findings indicate that during aversive interoceptive stimulation, there is quite some unexpected variability in startle responding that does not uniformly fit the usually observed pattern of exteroceptive fear potentiated startle. Known modulatory influences on startle that could explain the results are arousal and orientation of attention. Alternative explanations for the observed startle refer to the defense cascade model of Lang and colleagues, and the perceptual-defensive-recuperative model of Bolles and Fanselow.

Erik Ceunen

Onaangenaamheid en Vrees in Interoceptie

Proefschrift aangeboden tot het verkrijgen van de graad van Doctor in Psychologie (PhD), 2015
Promotor: Prof. Dr. Ilse Van Diest; copromotor: Prof. Dr. Johan W.S. Vlaeyen

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Interoceptieve vrees (IV) is de angst van lichamelijke sensaties. IV is relevant in aan aantal van psychiatrische en somatoforme stoornissen, in welke geleerde vrees van lichamelijke sensaties verondersteld wordt een belangrijke rol te spelen. Hoewel IV Conditionering (IVC) van belang is in het vergroten van begrip omtrent het ontstaan van deze stoornissen, is er tot heden nauwelijks onderzoek gedaan naar de basale vrees respons topografie t.a.v. experimenteel geïnduceerde interoceptieve sensaties.

Vrees in mensen kan gemeten worden d.m.v. zelfrapportage naar subjectieve vrees, onaangenaamheid, opwinding, gevoel van controle (dominantie) en de mate waarin men iets onaangenaam verwacht (expectancy). Een gevalideerde psychofysiologische maat voor motivationele intensiteit, d.w.z. opwinding, is huidgeleiding. De startle reflex wordt gemoduleerd door motivationele richting (aangenaam vs. onaangenaam) en kan als maat daarvan gebruikt worden. Tijdens onaangenaamheid en vrees is startle in het algemeen gepotentieerd in vergelijking tot neutrale en aangename toestanden.

Het hoofddoel van dit doctoraatsproject was om te achterhalen of het op de mens van toepassing zijnde startle paradigma gebruikt kan worden om defensieve responsmobilisatie bij aversieve interoceptive stimulatie te meten. Hiervoor beroepten we op een tweezijdige aanpak: enerzijds werd het concept interoceptie kritisch beschouwd, en anderzijds werden een reeks empirische studies uitgevoerd.

De review van interoceptie achterhaalde de ontwikkeling van het concept tot zijn moderne betekenis. De review stelt voor om interoceptie te beschouwen als de fenomenologische perceptie van de staat van het lichaam, ongeacht hoe deze perceptie ontstaat en ongeacht van diens accuratesse. Labels worden gesuggereerd voor specifieke componenten van interoceptie om ons toe te staan sensaties te classificeren als gelijkaardig of verschillend.

In de eerste studie gingen de deelnemers zitten in flexie, rechtop en extensie. De redenering achter de studie was dat houdingen emoties kunnen beïnvloeden en aldus mogelijk ook de startle. Bovendien zijn er eerder verschillen in startle geobserveerd tussen gastro-intestinale (geassocieerd met flexie) en respiratoire (extensie) stimulatie. Studie 1 vond dat de magnitude van de startle groter was tijdens extensie, vanwege de negatieve affectiviteit die gepaard ging met die houding.

In een tweede studie werden gezonde deelnemers blootgesteld aan de cold pressor test (CPT), ademen met weerstand, en inademing van met CO_2 verrijkte lucht. Startle amplitudes tijdens interoceptieve stimulatie waren lager dan tijdens stimulatievrije perioden. Respiratoire stimuli gingen gepaard met een lineair dalende lijn tijdens aangehouden stimulatie, terwijl de CPT een kwadratisch patroon ontlokte.

In de derde studie namen we bij vrouwen een potentiatie van de startle waar tijdens pijnlijke slokdarmstimulatie, maar niet bij mannen. In de vierde studie gebruikten we slokdarmstimulatie om een homoreflexief interoceptief conditioneringsparadigma vast te leggen. Daarin ging een niet pijnlijk slokdarm stimulus (geconditioneerde stimulus, CS) vooraf aan een pijnlijke slokdarm stimulus (ongeconditioneerde stimulus, OS) bij een gepaarde groep, maar niet bij de ongepaarde groep. Deelnemers in de gepaarde groep leerden de CS te vrezen, wat duidelijk werd uit hun OS-expectancy en huidgeleidingsrespons. Vrouwen die in de gepaarde groep zaten toonden ook een trend naar sterkere startles in reactie op de CS (in vergelijking met een veilige periode). Deze respons verdween weer tijdens de extinctiefase; bij mannen in de gepaarde groep daarentegen was er tijdens extinctie een grotere startle amplitude tijdens CS.

Samengevat wijzen onze resultaten er op dat er tijdens onaangename interoceptieve stimulatie een redelijk onverwachte variabiliteit is in de startle, die niet geheel overeenstemt met het patroon geobserveerd bij exteroceptieve vreespotentiatie. Gekende modulerende invloeden op

startle die de resultaten kunnen verklaren zijn opwinding en aandacht. Alternatieve verklaringen voor de startle bevindingen maken verwijzing naar het defense cascade model van Lang en collega's en het perceptueel-defensief-recuperatief model van Bolles en Fanselow.

A word of thanks…

They say life is a journey. The time as a PhD student is also a journey. At least it was for me.

We've all heard the cliché that journeys are not about the destination. And it's true: these years as a PhD student were not just about reaching the point where I am now, with a dissertation in hand and an upgrade from MSc to PhD. Yet I have to admit it does feel d@mn good to finally get here!

The many 'destinations' of life's journeys are primarily hallmarks that symbolize the end of a period. And what's life without hallmarks? What's life without such moments where we cannot yet see what's lying ahead, but where we need to stand still for a moment to reflect back on the journey that was?

Looking back, anyone can see I've taken a bit of a quirky path. I have made some choices in my academic career - temporarily deviations – that I consider part of my journey. At most these raised some questions and frowns. But no one ever stopped me, and I appreciate that. Everybody has to walk their own path to get to their destination.

What matters is that I got where I have to be. What matters are the people that are still here with me. What matters are those I've met during my journey, as well as those that set me on my journey.

What matters are those that were, are and/or will be there for me, support my choices, and don't judge me for it. What matters are those people I share memories with, and the people I value. What matters is

YOU.

Ilse, het is allemaal begonnen met een e-mail naar jou, lang, lang geleden. En hier zijn we dan, zo veel jaren later. Ik ben echt blij dat je mijn promotor bent geweest. Ik had het gevoel dat je vertrouwde op mijn kunnen (veel meer dan ikzelf) en dat je er altijd was wanneer ik dat nodig zou vinden. Alle studies zijn door jouw input in goede banen geleid vanaf het begin. Ik weet niet of ik je al gezegd heb dat ik op prijs stel hoe je feedback gaf op mijn schrijfsels: gedetailleerd, constructief en niet alleen duidend op wat er verbeterd kon worden, maar ook telkens benadrukkend wat je er goed aan vond. De laatste weken voor het indienen heb ik het je wel heel erg druk gemaakt. Na al die jaren op mijn gemak door mijn doctoraat te rijden aan gemiddeld 50km/h, werd het opeens duidelijk dat ik ook een turbomotor heb. En dan meteen ook nog maar wat nachtelijke pit stops overgeslagen. Ik hoop dat dit doctoraat niet alleen voor mij, maar ook voor jou een beetje een beloning is voor je eeuwige geduld.

Johan, ik weet nog goed dat jij mij het telefoontje gaf dat voor mij ver boven de meest positieve arousing stimulus van het International Affective Digital Sounds (IADS) uitstak: het telefoontje waarin je me liet weten dat ik in Leuven kon beginnen. (Slechts 1 ander telefoontje heeft dit ooit overtroffen.) Als copromotor kwam je op de proppen met allerlei suggesties, maar liet me de vrijheid te kiezen welke te volgen, en welke niet. Ik onthoud nog steeds dat ik mijn allereerste publicatie[1] te danken heb aan zo'n suggestie van jou. En natuurlijk was ook mijn doctoraatsproject enkel mogelijk dankzij het fonds van het *FWO* voor het Odysseus programma 'pain and disability research program' dat je hebt weten binnen te halen.

Meike, Seneca's woorden "Omni fine initium novum" lijken mij hier gepast. Jouw bevindingen met respiratoire stimuli vormden de inspiratie voor mijn project en je keuze voor het woord interoceptie spoorde me aan uit te werken wat dit concept voor mij betekent.

[1] als eerste auteur

Steven, jij hielp me op weg tijdens mijn eerste experiment. Het leek toen een berg aan belangrijke details om te onthouden en alsof zo veel dingen fout konden gaan. Maar al snel bevond ik me in jou plaats met het begeleiden en geruststellen van nieuwe onderzoekers.

Natacha en *Katja*, zonder jullie bijdrage waren de laatste twee studies nooit mogelijk geweest, en de perfecte gender balans in de allerlaatste studie al helemaal niet. Bovendien nooit iemand flauw gevallen, desondanks dat wij de meest invasieve stimulus in het PSI hadden. Jullie leveren het bewijs dat informele ontvangst en uitleg op een vriendschappelijke toon net zo goed gestandaardiseerd kan zijn over alle deelnemers heen (ook al lijkt het voor de deelnemers heel spontaan): iets waar ze op Gasthuisberg door ervaring al langer de voordelen goed van inzien lijkt me.

Giao en *Nathalie Weltens*: ook dank aan jullie voor de initiële begeleiding van Natacha en Katja.

Lukas, jij was al van in het prille begin betrokken bij mijn project: bij mijn sporadische bezoekjes aan Gasthuisberg ervoer ik je altijd als heel betrokken maar gelijk ook heel relaxed: altijd fijn om mee samen te werken.

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Jonas, mijn data geef ik niet graag uit handen, maar wat ik van je terugkrijg weet me te overtuigen voor jou een uitzondering te maken.

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The members and chairman of the guidance committee (*Omer, Lukas, Hans Op de Beeck, Geert Crombez, Hartmut Schächinger,* and *Rudi D'Hooge*): I carefully noted the constructive feedback and questions which you provided two years into my PhD. Even after all the time that has passed since then, I have still referred back to these notes when writing the introduction and discussion section of this dissertation, so thank you.

Hans, Koen Schruers, and *André Schulz*: as opponents you will be among the few people, if not the only ones to read through this entire dissertation from beginning to end. (In contrast to the remaining majority who will probably just judge the cover I designed, and limit themselves to reading no further than the acknowledgments if reading anything at all.) I'm immensely thankful for your effort and time, and am honored that you have agreed to be in my jury. And thank you *chairman*!

Marko Jelicic, het begon eigenlijk allemaal tijdens mijn master stage, waar ik mijn eerste (min of meer) zelfstandige onderzoekservaring opdeed als jouw thesisstudent, wat me uiteindelijk heeft geleid tot waar ik vandaag ben (en ook nog een publicatie opleverde).

Also a very special thanks for *Stefan Sütterlin* for providing an opportunity to continue my academic career at least a little while longer, and to meet kind new colleagues. And of course to *Claus*: without you this book would not yet have materialized at this time. Thanks for using applied psychology in my own advantage and unknowingly introducing me to the positive side of Parkinson's Law[2].

[2] *work expands to fit the time allotted*, meaning it takes as long to finish something as the time you give yourself to finish it.

Jeroen, Johan Hendrickx, Martine Haesendonk, Liesbet, An Van Kets, Armand, Luc Kelders, Claudia: ieder van jullie heeft zijn of haar eigen unieke praktische bijdragen geleverd voor mij tijdens mijn tijd aan de KU Leuven. Jullie mogen gerust zijn: dat vergeet ik niet zo maar!

Fellow researchers, apart from the more formal professional interactions such as in labmeetings, retreats and international conferences, I will mostly remember you as during time spent together informally. Especially for doing the not so serious stuff like joining me in making and throwing a huge paper airplane made out of a discarded science poster; I'll remember the one who pulled a prank where the keys of another colleague's keyboard got switched (I wish I knew how, primarily just to be able to save myself if that ever happened to me); I'll remember who was always available giving tips on the last practical steps in finishing this; all of you for playing a team building game that temporarily (I hope not permanently) undermined trust in each other (at least if you play it like a child that just wants to win – guys! And yes, I'm guilty too, but hey: a game is just a game); and of course that time during a dance workshop were most of us were trying our best to not look ridiculous (ha!), enjoy and just go with the flow. And not to forget: the potluck food fests, a.k.a. international dinner parties (thank you for hosting these, *Angela* and *Omer*).

Katleen, Wan Li, Elke, Thomas, as well as *Johan Bresseleers* and *Stien*: together with Meike, Steven and some others who had already moved on before my arrival, you have all paved the way for me and those after you.

I realize all too well our group should also be grateful for having its perspectives and understanding widened thanks to *Karel Van de Woestijne, Ann, Sibylle, Martien Schrooten, Daniel Vigo, Stefan, Hassan, Nicole, Holly,* and *Andreas*, joining in from other research groups and even other fields of study.

Kim, Tom, Elyn, Ali, Stéphanie, Elena, Elham, Barbara, Rena, Ruth, Mathias, Nele, Marta, Joanna, Nathalie Claes, Farah and *Maaike*: although our paths will go different ways, I feel privileged to remember you in part as during our early careers here at KU Leuven in this decisive period in our lives.

Also, forever in my memory as integral part of my time as a PhD student will be the daily early morning trainings with **羅老師** (you made me feel overly praised in a good way) and with my friends *Olivier, Sofie, Zhaoyin, Karlien, Sam*, and other fellow early bird students. Actually, my training became so important that it was not only part of my mornings, but also my holidays in mainland China during my years as PhD. Thank you **李老**师, a second very 厉害 80 year old **李老**师, **姚老**师, and 尹**老**师 for your eagerness to share your knowledge and to 'transfer' your skills to me. I'm also very honored to have 暎渲 as my 师姐 and to have met the now late master 周靖轩.

On top of all that training, I had the quirk in my PhD journey that I referred to earlier: this quirk was a sabbatical training year which –from my perspective - was also part of my time as a PhD student. Thank you 黄老师, but also thank you to 小李 and 小许, and also 小童， 小韩 and others for teaching me.

And a big thank you for a lot of the aforementioned as well as other people in China for their kindness, welcoming me in their life, and sometimes even in their residence. 谢谢你们!

Grote broer, grote zus: zou het kunnen dat ik door vroeger altijd achterop te hinken qua leeftijdsfase en dus door achterop te hinken qua leeftijdsgebonden mentale vaardigheden, nu nog altijd aan het overcompenseren ben? Als dat het geval is, dan heb ik deze prestatie aan jullie te danken! ;) Anderzijds,

ik wou als kind altijd al professor worden, maar dat was dan eerder omdat ik dan dingen zou kunnen maken die aan het magische grenzen, zoals tijdsmachines e.d.…

Pa, ma. Met de paplepel heb ik jullie als voorbeelden binnengekregen: enerzijds nadruk op het belang van lichamelijke ontwikkeling (een prijzenkast zo vol als bij ons thuis leek mij lange tijd iets normaal), en anderzijds op intellectuele nieuwsgierigheid (ook een eigen boekenkast vind ik onmisbaar). Mens sana in corpore sano. Verbaast het dan eigenlijk nog dat ik in de groep gezondheidspsychologie terecht gekomen ben? Het vakgebied waar zowel lichaam als geest beiden een belangrijke rol spelen? Ik ben ook de fietstochtjes nog niet vergeten, die mij hielpen te ontstressen toen ik mijn eerste witte haartjes kreeg helemaal aan het begin van mijn doctoraat. Er is zoveel om jullie voor te bedanken, maar deze doctoraatsthesis moet over mijn onderzoek gaan, en daar gaat normaal geen uitgebreide autobiografie aan vooraf dus ik kan alles waar ik jullie dankbaar voor ben onmogelijk allemaal opsommen.

En dan nog een heel belangrijk “me vrouwtje”. Als je al zo lang samen bent, dan is dat helaas niet elke seconde in perfecte harmonie. Ik wil je daarom een ding op het hart drukken en dat is dat ik je heel erg dankbaar ben en zal blijven. Jij hebt mij op het idee gebracht en aangespoord om na mijn master verder te blijven gaan in de academische wereld. Jij hebt mij ook gepusht om door te zetten toen ik het tijdens mijn doctoraat een tijdje niet meer zag zitten en even een beetje te veel last had van imposter syndrome. En vooral: jij hebt van mijn tijd als doctoraatsstudent een echte rite-of-passage gemaakt gevuld met wel héél veel ‘significant life events’. De doctoraatsopleiding is zo als het ware slechts een raamvertelling die al deze belangrijke stappen in het leven met elkaar verbindt. Dankzij jou kan ik op deze periode in mijn leven terugkijken als een periode van persoonlijke groei.

Duiveltje, op een dag lees je dit misschien en besef je hoe dankbaar ik ben voor de uren na mijn werk en de weekends - wij hebben samen echt veel liggen spelen en babbelen, ook al ga je daar waarschijnlijk niet veel herinneringen aan overhouden. Het was en is nog altijd precies of ik een betere versie van mezelf zie opgroeien, maar ik besef ook wel dat jij jij bent en niet mij. Ik kan alleen maar zeggen dat jij het beste in mij naar boven haalt en me aanzet dat te blijven doen. Ik hoop dat ik jou altijd zo gelukkig kan maken als jij mij.

Familie, schoonfamilie, friends, and any readers that didn’t get mention above: I appreciate you for who you are to me.

Thanks everyone! 谢谢大家！ Viele Dank an alle! ευχαριστώ όλους! ¡Gracias a todos! Dziękuję wszystkim! امش زا رکشت اب! Спасибо вам всем! Gratias vobis ago! Dank je wel allemaal!

It definitely was a memorable and meaningful journey for me. Before I move on to the next, I’ll leave you this dissertation[3], which you can consider as my token of gratitude and as a symbol of my journey.

- Erik -

[3] Digital copies freely available online and on request! :)

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Part I

General Introduction

CHAPTER 1

On the origin of interoception

Ceunen, Vlaeyen & Van Diest (In preparation)

Abstract:

Over the course of a century, the meaning of interoception has changed from the restrictive to the inclusive. In its inclusive sense, it bears relevance to every individual via its link to emotion, decision making, time-perception, health, pain and various other areas of life. While the label for the perception of the body state changes over time, the need for an overarching concept remains. Many aspects can make an interoceptive sensation unique and distinct from any other interoceptive sensation. This can range from the sense of agency, to the physical cause of such a sensation, the ontogenetic origin, the efferent innervation, and afferent pathways of the tissue involved amongst others. In its overarching meaning interoception primarily is a product of the central nervous system, a construct based on an integration of various sources, not per se including afferent information. This paper proposes a definition of interoception as based on subjective experience, and pleas for the use of specific vocabulary in addressing the many aspects that contribute to it.

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1. Introduction

While interoception is a term that has gained and still is gaining popularity in the academic literature since the start of the millennium, consensus on its meaning is as yet not fully established. What is generally agreed upon by most current scholars is that interoception is the perception of the state of the body. The exact interpretation of this definition ranges from the original restrictive meaning which is still adhered to by some (e.g., Dworkin, 2007a) to the now more commonly used very inclusive meaning (e.g., Craig, 2002; Wiens, 2005). The restrictive meaning holds that only sensations stemming from viscera are interoceptive. However, in this thesis, the author uses interoception in its inclusive sense; as an umbrella term for the phenomenological experience of the body state, an experience which is ultimately a product of the central nervous system *(CNS)*, regardless of what information the brain uses and does not use to construct this experience. Arguments supporting this choice will be addressed and elaborated upon later throughout different parts of this chapter.

The relevance of interoception in its inclusive meaning will be illustrated by briefly highlighting its range of involvement across a spectrum of different areas of psychology and health. Next, the importance of proper communication on interoception will be stressed, regardless of the definition one has. The original meaning of interoception will be examined and a short overview of the linguistic development of interoception and related concepts over time will be provided. Finally, an impetus will be given for applying a clear vocabulary that allows to distinguish between the various aspects which can contribute to interoception, while retaining the use of an overarching term. This chapter will end with some concluding remarks.

1.1 Scope of relevance

Although not yet common parlance in medical circles, “interoception” is a concept which relates to a very wide range of health related and psychological aspects of human life, playing a role in every individual. As a consequence, interoception is of pivotal importance to a wide range of research, theory and translational applications of research findings. A cursory glance at the literature is sufficient to see that interoception relates to a vast range of subjects. These subjects include negative emotions (Pollatos, Schandry, Auer, & Kaufmann, 2007); anxiety, anxiety disorders and affective disorders (Barlow, Allen, & Choate, 2004; Domschke, Stevens, Pfleiderer, & Gerlach, 2010; Dunn, Stefanovitch, et al., 2010; Paulus & Stein, 2010; Stern, 2014), medically unexplained symptoms (MUS) (Bogaerts et al., 2010; Schaefer, Egloff, & Witthöft, 2012) as well as medically identifiable symptoms (Janssens, 2011; Julius, Davenport, & Davenport, 2002; Mandelzweig, Goldbourt, Boyko, & Tanne, 2006), pain (Craig, 2003), emotions in general (Craig, 2008; Damasio, 1994; Damasio & Carvalho, 2013; James, 1884; Lange, 1885; Schachter & Singer, 1962; Wiens, 2005; Zaki, Davis, & Ochsner, 2012), emotion regulation (Füstös, Gramann, Herbert, & Pollatos, 2012), decision making (Bechara, Damasio, Tranel,

& Damasio, 1997; Clark et al., 2008; Damasio, 1994; Dunn, Evans, Makarova, White, & Clark, 2012; Dunn, Galton, et al., 2010; Paulus, 2007, 2011), subjective time perception (Craig, 2009; Pollatos, Laubrock, & Wittmann, 2014), subjective (self)awareness and consciousness (Craig, 2004; Seth, Suzuki, & Critchley, 2011), food and water intake (Berthoud, 2006; Brannigan, Stevenson, & Francis, 2014; Herbert, Herbert, et al., 2012), eating disorders (Herbert & Pollatos, 2014; Pollatos et al., 2008), addiction (Naqvi & Bechara, 2010; Paulus, Tapert, & Schulteis, 2009; Verdejo-Garcia, Clark, & Dunn, 2012), sexual functioning (Everaerd, Both, & Laan, 2006; Gerbarg & Brown, 2011), empathy (Fukushima, Terasawa, & Umeda, 2011; Singer, Critchley, & Preuschoff, 2009), meditation (Farb, Segal, & Anderson, 2012), hypnosis (Woody & Szechtman, 2007) and of course interoceptive conditioning (Ceunen, Zaman, et al., in preparation; Pappens et al., 2013; Razran, 1961).

Although this list is unlikely to be exhaustive, and although it is beyond the scope of this chapter to specify for each of these subjects how they relate to interoception, it should be clear that interoception is not to be considered a minor field of study within psychology and health, and that its study has widespread relevance. Although none of the studies described in later chapters in this dissertation have any immediate clinical relevance, all studies conducted in the context of the doctoral project do relate back to one or more of the aforementioned subjects; the studies primarily relate to emotion, negative affective states, pain and interoceptive conditioning. This relation will be elaborated upon in later chapters, as the focus of this chapter is to address the semantics of interoception.

One aspect that already deserves mention though, is pain. Pain has been intentionally induced in three quarters of the studies. This necessitates a clarification of how pain is considered in relation to interoception in this dissertation. The international association for the study of pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey, 1986). While those with a narrow definition of interoception would only consider visceral pain interoceptive, those with more inclusive definitions of interoception such as the one used throughout this dissertation- consider all forms of pain as a form of interoception. This inclusive approach is supported by functional neuroimaging studies which find that the neural network activated during pain and during other forms of interoception are very much the same (Legrain, Iannetti, Plaghki, & Mouraux, 2011; Moseley, Gallace, & Spence, 2012). It is also indirectly supported by the well-validated finding that emotion plays a significant role in pain; a role which it also plays in all other forms of interoception. For example, negative emotions impact the affective component of pain negatively and decrease pain tolerance (Carter et al., 2002), while positive emotion increases pain tolerance (Zweyer, Velker, & Ruch, 2004). In dyspnea, a non-painful but also unpleasant interoceptive sensation otherwise described as breathlessness, one can observe

something remarkably similar, with more respiratory symptom report in a negative affective context than in a positive affective context (von Leupoldt & Dahme, 2013). This can be taken as an extra indication that pain really is nothing more than a specific form of interoception, but interoception nevertheless. Regardless of which definition of interoception one applies, the inclusion of pain in the definition of interoception –even if merely including visceral pain- has its merit. It provides a fertile soil for cross-fertilization of ideas from the vast area of pain research on the one hand, and that of research on non-painful types of interoception on the other.

1.2. Communicating on interoception

It is surprising that communication around the concept interoception often stumbles over definitional differences between authors. While both an overarching concept of body perception as well as more specific concepts deserve their own place, the exclusive focus on one approach could lead to undervalue the importance of the other. For example, an overarching concept has as one advantage that it crosses the bridges between different types of research findings. How this overarching concept is labeled changes over time as do so many aspects of language: in the 19th century it was referred to as "coenaesthesis", in the early 20th century "coenesthesia", in the second half of the 20th century "somesthesis", and now in the 21st century it is most frequently referred to as "interoception" (see section 2.2). Regardless of its label, an exclusive focus on an umbrella term may lead to premature overgeneralization of findings.

For example, the accuracy with which to detect heartbeats has long been referred to as a general measure of overall interoceptive accuracy. Although it is true that interoception includes the ability to perceive heartbeats, prior to having tested how accuracy with which to detect heartbeats correlates with accuracy of perception of the heterogeneous plethora of other types of interoceptive sensations, it would be premature to say that all forms of interoceptive accuracy are poor in those who have poor accuracy in perceiving their own heartbeat - even if this hunch later appears to be correct. Such a conclusion would remain premature until the point where there are actual findings which support the conclusion that heartbeat accuracy can be generalized to reflect accuracy of perception of other interoceptive sensations. This caution has also been emphasized by the author previously (Ceunen, Van Diest, & Vlaeyen, 2013). After submission of this manuscript cautioning against premature generalization, Herbert and colleagues published a research article which does suggest that accurate cardioceptive perceivers also have more accurate perception of sensations that aid in the regulation of water intake (Herbert, Muth, Pollatos, & Herbert, 2012). Even when there finally are findings like these which allow us to gradually make evidence-based extrapolations, and which stimulate us to speculate on how generalizable findings are, we need at the same time remain

careful not to overgeneralize beyond what has been investigated, nor to blindly accept extrapolations suggested by others as facts.

Arguments referring to neuroanatomy are sometimes used to justify generalizations such as these, for example by referring to the convergence of sensory information from distinct bodily organs and of distinct types of visceroceptors in the Nucleus of the Solitary Tract (NTS). Though processed in very close proximity to each other within the NTS and sometimes in overlapping areas, the respective loci for each of these sensations within the NTS are still somewhat distinct (Paton, Li, & Kasparov, 1999). Also, convergence at one site of processing does not necessarily mean that at all other levels of processing there is not a single distinction between any of these distinct body state sensations. Therefore, to prevent oneself from being tempted into premature generalization, it is useful to keep in mind that different sensations not only overlap in some aspects (e.g., all being interoceptive), but are also unique in their own ways. This holds true even if all these sensations have in common that they refer to the body state and have at least one or more common processing sites in the brain. Examples of levels at which sensations can be considered as being distinct from one another can be: whether sensations are entoperipheral or epiperipheral in origin; whether they are exogenous or endogenous; whether the individual experiences a sense of agency over the sensations or not; whether the sensations stem from tissues with efferent autonomic nervous system (ANS) and enteric nervous system (ENS) or from somatic nervous system (SNS) innervation; whether the concerned organs are identical or different in their ontogenetic origin as coming from ectoderm, endoderm, or mesoderm; and which afferent homeostatic pathways and basal processing structures are involved in the early processing. I will go into more detail on a number of these in separate sections later on (see section 3.1, 3.2, and 3.3). The main point is that, even though there may be commonalities between different body state sensations, there are also always differences. Interoceptive sensations are characterized by both specificity and convergence. Though an overarching term can be beneficial, we should always keep in mind to avoid premature generalization that has not first been specifically addressed in research.

On the other end of the spectrum, as opposed to the exclusive focus on an overarching concept and an exclusive focus on similarities, there can be an exclusive restrictive focus on one of the subcomponents of interoception; on what makes this subcomponent different from other sensations. Just as the broad focus on similarities, so too does the narrow focus on differences have its own advantages and disadvantages. An advantage of research with a narrow focus is that this will provide specific information on a subcomponent of interoception, which can then be contrasted to other subcomponents of interoception, which allows us to make statements on the generalizability or specificity of these and other findings. A disadvantage of a more narrow focus is that at times, findings

from certain research areas do not provide nor receive input from distinct yet related research areas, thus the information from different types of studies is less likely to be put together to form a completer picture.

To allow for better communication of not only findings, but also for cross-fertilization in the sharing and forming of ideas and insights on interoception, and on its relation to human psychological faculties and health related issues, there needs to be a common understanding amongst researchers. To achieve this, it is imperative that the various components of the concept interoception are outlined so they become more generally acknowledged as distinct, individual aspects which each deserve their own specific labels, while at the same time there remains a concept which integrates all of these aspects. That labels are prone to change over time, that they differ between certain research areas, and that there are individual differences in the use of these labels only makes an outline of this topic more relevant. Although this chapter is not written on the pretense of being able to create a universally accepted consensus on which labels to use, it does intend to at least provide an impetus for the use of distinct labels for the distinct aspects of interoception that will be covered here. Moreover, this chapter emphasizes that it is every author's duty to introduce their own however short definition of interoception for each individual publication, and to make sure it matches with how they use the word throughout that publication.

2. History of a concept

2.1. Etymology

To come to a deeper understanding of the meaning of a word or concept, it is customary to refer to its origins and then address whether, and if so, how its meaning has changed over time. Interoception is a relatively recent concept which arose together with the concepts proprioception and exteroception during the early 20th century. The first known usage of the concept interoception in publication dates to 1906 by Charles S. Sherrington in his book "The Integrative Action of the Nervous System"(Sherrington, 1906), which is a collection of lectures he had given at an unknown date prior to publication. In the book, Sherrington talks of "interoceptors", "interoceptive receptor fields", "interoceptive reflex arcs", "interoceptive surface", and "interoceptive segments". Interestingly, at this point in time, the noun "interoception" itself was not yet introduced in publication. In fact, it is only in the 1940's that the word "interoception" first appears in scientific journals (Airapetyantz & Bykov, 1945; Freeman & Sharp, 1941; Mogendovich, 1941). Regardless, we do need to refer back to Sherrington to understand the original meaning of the *concept* interoception.

Sherrington referred to the internal surface of the body as interoceptive, as opposed to exteroceptive which he defined as the external surface in direct contact with the environment. In this

meaning interoceptive then can be considered a synonym for things entoperipheral, while exteroceptive is a synonym for things epiperipheral. Thus, according to this definition, cutaneous sensations would be considered exteroceptive sensations, but subcutaneous not. In the vast entoperiphery, Sherrington further distinguished between deep somatic tissue, i.e. skeletal muscle, as a site specific to proprioceptors, and the viscera as site specific to interoceptors. Furthermore, he considered not only perception of light, sound, odor, and mechanical touch as exteroceptive, but also perception of temperature and nociception. The inclusion of temperature and nociception in the definition of exteroception, contrasts to these sensations being included in more recent definitions of interoception, such as the one put forward by Craig (2002). In Sherrington's 1906 definition, what distinguished interoception (and proprioception) from exteroception is that only the latter possesses the quality of projicience. Projicience is a term which he used to refer to two aspects: (1) the perception of something at a distance and (2) projection in the sense of estimating the future based on what is happening now. In other words, Sherrington labeled perception of precurrent exogenous stimuli as exteroceptive, while sensations of endogenous origin as either proprioceptive or interoceptive, depending on whether they arise in respectively skeletal muscles or viscera.

While the linguistic contraction of "interior *receptor*" was the basis for "interoceptor" and by extension the adjective "interoceptive", in contrast the noun "interoception" was first introduced more than a third of a century later, and can either be taken to be a variation of the contraction of "interior receptor", or to be a new contraction, namely "interior *perception*". Whatever interoception's original meaning, modern day use of "interoception", and to some extent "interoceptive" have generally come to refer to the broader phenomenological *perception*, rather than to refer merely to location and stimulation of receptors. In other words the focus of the concept has shifted from referring solely to the afferent relay of receptors of the ANS, to becoming a word which is now most frequently used as an umbrella concept for a multi-sensory, multimodal integrated percept of the body state.

2.2 Semantic evolution

In order to identify the frequency and evolution of usage of specific interoception related labels, an extensive search in Google Scholar was performed. The inclusion of selected terms for which a frequency of occurrence was obtained, was motivated by the idea that at some point in time all of these included terms have had a nearly synonymous meaning to one of our primary three search entries: interoceptor, interoceptive, interoception. In addition to these three words, the search entries included the following: viscероceptor, visceroceptive, visceroception, somesthesis, somesthetic, somesthesia, coenesthesis, coenesthetic, and coenesthesia. The frequency of each of these words was established by identifying the number of search results from 1800 up to and including 2010, with the

"include patents" and "include citations" boxes unchecked. The number of hits per word was assessed per period of five years (1901 up to and including 1905, 1906 – 1910, 1911-1915, etc.), excepted the period from 1800 up to and including 1900, a period for which the author did not deem it necessary to subdivide into smaller time periods and which the author thus took as a whole.

Moreover, the same search procedure was conducted for variations of each of these words to allow for identifying possible changes in spelling preference over time and to identify the first introduction of alternate spellings. The author identified and conducted a separate search for alternate spellings, which were: intero*re*ceptor; intero*re*ceptive, intero*per*ceptive; intero*re*ception, intero*per*ception; viscero*re*ceptor; viscero*re*ceptive, viscero*per*ceptive; viscero*re*ception, viscero*per*ception; somaesthesis, somataesthesis, somatesthesis; somaesthetic, somataesthetic, somatesthetic, somathestetic; someaesthesia, somataesthesia, somatesthesia; caenesthesis, caenaesthesis, coenaesthesis, coenoesthesis, cenesthesis, cenoesthesis; caenesthetic, caenaesthetic, coenaesthetic, coenoesthetic, cenesthetic, cenoesthetic; caenesthesia, caenaesthesia, coenaesthesia, coenoesthesia, cenesthesia, cenoesthesia.

Because Google Scholar identifies and includes some alternate spellings or concepts automatically in the search results it produces, this could potentially create the problem of getting wrong estimates. This problem was bypassed by entering each individual search entry between brackets so only hits for the specified spelling resulted. Another aspect taken into consideration is that in Google Scholar, when the number of results is higher than 10, the total number of hits as indicated at the first page of search results is usually merely an initial approximation by the Google Scholar search engine, but does not always correspond exactly to the total number of hits. The correct number of hits is indicated on the last page of results (if the total is less than approximately 950 hits). Therefore, in order to get a more accurate approximation of the exact amount of hits, the total number of hits as indicated on the last accessible page of results was used. For those hits ranging over 1000 with 10 hits per page, Google Scholar does not display pages beyond approximately the 95th page, so approximations of the total number of hits when there are more than approximately 950 results in total may be less accurate.

Although it is beyond the scope of this chapter to present the collected data in extensive detail, a selection has been made of aspects which stand out and provide an interesting perspective on the development of word preferences (See Table 1). During the entire span of the 19th century, interoception was not yet an existent word. Instead, with a total of 220 results for its various spellings in that period, 'coenesthesis' was by far the most popular word which comes closest to the inclusive meaning of interoception, followed in popularity by 'coenesthesia', which in its various spellings totals only 11 results for that same period. While 'somesthesis' and 'somesthesia' appear to be non-existent

in publication during the 19th century, the adjective 'somesthetic' did exist in publication starting around the late 1800's (totaling 5 results up until 1900). Bailey (1906) was responsible for introducing the word 'somesthesis' into the English language, in the same year that Sherrington (1906) published the first work to use the words 'interoceptor' and 'interoceptive'. Two years later, it was again Bailey (1908) who first introduced the word 'somesthesia'. About a century later, in the period from 2006 up to and including 2010, 'somesthesis' and 'coenesthesia' are still relatively popular nouns, with respectively 284 and 263 publications in which these words appear, but both are very much overshadowed by the popularity of the noun 'interoception' with mention in 1745 sources. It is true that in that five year period from 2006 up to and including 2010, the adjective 'somesthetic' also occurs in a large number of sources (1579 sources to be precise), but this seems to be largely due to the use of the adjective as a synonym for 'somatosensory' when referring to the CNS areas SI and SII. Regardless of the reason for its popular use, the occurrence of the adjective 'somesthetic' in recent years is still by far outdone by the adjective 'interoceptive', the latter which occurred almost five times as much as the former, in a total of 7471 sources.

Table 1.

Word preferences over the centuries.

	Year of first mention (author)	Most popular time relative to 'equivalent' words	Publications from 2006 to 2010
Interoceptor	1906 (Sherrington)	1906-2010	202
Viscероceptor	1948 (Strong & Elwyn)	/	18
Interoceptive	1906 (Sherrington)	1981-2010	7471
Visceroceptive	1952 (MacLean, Horwitz, & Robinson)	/	170
Somesthetic	1900 (Taylor & Haughton)	1897-1900; 1906-1910; 1916-1920; 1931-1980	1579
Somaesthetic	**1897 (Barker)**		
Coenesthetic	1901 (Kellogg)	1858; 1901-1915	194
Caenaesthetic	**1858 (Noble)**		
Interoception	1941 (Freeman & Sharp; Mogendovich)	2001-2010	1745
Visceroception	1974 (Dutov)	/	103
Visceroreception	**1967 (Merkulova & Popova)**		
Somesthesis	1906 (Bailey)	1936-1940; 1946-2000	284
Somesthesia	1908 (Bailey)	/	205
Coenesthesis	1816 (de Hájnik)	1794-1910	53
Caenesthesis	**1794 (Hübner)**		
Coenesthesia	1817 (de Nyir)	1911-1935; 1941-1945	263

Alternative spellings are listed and marked with an asterisk for those words where the now most widely used spelling arose only later.

Although it is obvious how this data set can provide insights on the development of word usage, it may be unclear how it can shed light on the development of word meaning over the years, hence a clarification for the latter is in order. It has already been pointed out that in their initial existence, the words 'interoceptive' and 'interoception' were more narrowly defined concepts (Sherrington, 1906) (see section 2.1). In 2002 Bud A. Craig made a plea to consider interoception as a more overarching term (Craig, 2002). Since around that time, the usage of the word interoception has spiked in popularity. Moreover, as that paper has been cited in well over 2000 other publications, it is

safe to assume it has not gone unnoticed. If we then reconsider the collected word prevalence data, it appears as if interoception is most frequently used from the point in time onward when a broader meaning was first attributed to it by Craig. The increase in popularity after this point onwards may be attributed to at least two important factors. First, an initial suggestion towards a conceptual shift likely leads to a lack of consensus and thus increased mention and use of the word in attempts to reach consensus, or in attempts to think through, clarify and solidify its meaning. Secondly and most importantly, concepts with broad meanings have broader relevance to a variety of research lines, whereas concepts with narrow meanings have relevance to a more limited number of research areas. Following this logic, we can assume that words increase in popularity at least in part because their meanings shift to refer to a broader concept. (However, we cannot conclude the reverse: that when words decline in popularity, it is because their meanings have narrowed down. It is possible for words to decline in popularity simply because other words are attributed a similar meaning and have become more popular.)

The arguments outlined here justify two choices made in this thesis. First and most important, is that a choice has been made to adopt and extend on the use of the word interoception in its broad, overarching meaning, rather than try to revert back to its originally restrictive meaning. In other words, this thesis builds on the already existing conceptual change that has occurred after the original inception of the words 'interoceptive' and 'interoception' last century. Secondly, given that currently interoception is the most widely used word from all previously indexed, related concepts, it justifies the choice for the title and focus of this thesis to be on the words interoception and interoceptive to refer to the broader perception of the body state, and to use related definitions to more specifically classify sensations.

3. Aspects of interoception

While this chapter argues in favor of using the word interoception as an overarching concept, it also pleas that anything which falls within this larger concept, or which is related but different, ought to be labeled differently and more specifically in order to avoid confusion as well as to allow for more effective communication. Whether there is consensus on the labels is only of secondary importance, as meaning attributed to labels will naturally evolve over time. Of primary importance is to establish a consensus that each of the concepts listed below deserve their own labels and are not to be confused with one another, even though they may be related to one another.

3.1. Exogenous versus endogenous origins

If something has an exogenous origin, this means that the source originates or is attributable to an agent outside the organism. If something is endogenous, it means it comes from within the organism

and is not attributable to an external agent. It is clear that 'exogenous' and 'endogenous' are antonyms of one another. Likewise, exteroception is commonly accepted to be the antonym of interoception. Therefore, how exteroception is defined, to some extent affects the meaning attributed to interoception. This can be somewhat problematic, as there has been a conceptual shift in the meaning of interoception, while the meaning of exteroception has hardly changed for most who use it. The resulting problem this poses for the definition of interoception is twofold.

The first problem relates to the meaning attributed to exteroception, namely that it is the sensory perception of exogenous stimuli. This meaning is often interpreted to mean that all sensations elicited by exogenous stimuli are exteroceptive, and considers the actual stimulus origin of primary importance and not the subjective perception arising in the CNS. This approach implies that any experimental set-up that intends to study interoception, would only be able to do so if sensations would have an endogenous origin. This would largely preclude the study of interoception and would have to discard many of the published studies on the topic, as nearly every stimulus applied in lab set-ups has an exogenous origin. In other words, confounding exogenous and endogenous origins with the phenomenological *perception* of something as relating to the surrounding environment or to the body state, would seriously set back the study of interoception, and conclusions made on the topic of interoception. Furthermore, many naturally occurring body state sensations are very frequently elicited by exogenous stimuli. For example, gastro-intestinal sensations can follow the ingestion of exogenous substances. Likewise, the sensation of feeling cold is not necessarily of endogenous origin as can occur with illness, but can just as well have an actual exogenous cause such as a cold ambient temperature. As the human body does not act in isolation of its surroundings, it is necessary to keep concepts that make a distinction between the origins of a stimulus (exogenous vs endogenous) distinct from broader concepts that make a distinction between different types of experiential perception as arising in the CNS (interoception vs exteroception).

The second problem posed by referring to exteroception as an antonym of interoception is that this often leads to the assumption that the receptor systems and pathways for both must by definition be mutually exclusive. However, that is not necessarily the case when using the inclusive definition of interoception. For example, seeing and feeling snow in the absence of cold sensations, can lead to the perception that the perceived snow, although not imaginary, is not genuine snow. This exteroceptive percept is the result from an integration of various sensory modalities including body state sensations (as well as past experience and other factors). If we imagine a nearly identical scenario but accompanied by sensations typical of physical illness, this can give a whole new phenomenological feel to the absence of cold sensations, where this absence can then be integrated in the interoceptive perception of the body state rather than the exteroceptive perception of the surrounding

environment. This example, though fictional, illustrates that exteroception and interoception can rely on identical sensory receptors and afferent pathways, and that they need not be mutually exclusive on any of the levels preceding the higher order processing of interoception and exteroception.

The main point of this section is that sensory origin or stimulus properties (exogenous vs endogenous) are not of relevance to determining whether a *percept* is interoceptive or exteroceptive when using the inclusive definition of interoception. What matters in the inclusive definition is whether in the phenomenological perception a sensation is perceived as informative about the body state or about the surroundings (see section 3.4). In those cases where the actual origin of a sensation is considered of relevance for research purposes or conclusions rather than the phenomenological perception, it is preferable to refer to the eliciting stimuli as exogenous or endogenous (whichever is applicable), and give preference to the use of these terms over ambiguous terminology.

3.2 Visceroceptor, visceroceptive, visceroception – a reference to efferent innervation

Although not in popular usage yet (see Table 1), in this chapter it is argued that there is a place for the words visceroceptor, visceroceptive and visceroception. These labels have become more suitable to refer to the once restrictive concepts that "interoceptor", "interoceptive", and "interoception" originally referred to, i.e. things specifically and solely pertaining to the viscera and nothing else (Sherrington, 1906). Such a distinction is necessary as interoception has come to adopt a more broad meaning, which refers to the integrated cross-modal CNS perception of the body state (Craig, 2008; Critchley & Harrison, 2013). Distinguishing between the broad concepts "interoceptive", and "interoception" on the one hand, and the narrow concepts "visceroceptive" and "visceroception" on the other, helps to avoid all possible confusion between the broad and the specific. Moreover, given the broad meaning of interoception, it can be argued that any receptor that can provide information to create a CNS representation of the body state can be considered an interoceptor, and not just those receptors in the viscera. This makes the word interoceptor so inclusive it becomes redundant ("receptor" would be sufficient). At the same time this necessitates the use of a more specific label for referring to only those receptors located in visceral tissue. Hence it is proposed here to refer to these visceral receptors as "visceroceptors" rather than "interoceptors". In the same vein the adjective "interoceptive" and the noun "interoception" should be solely reserved for more broad meanings pertaining to perception of the body state when further details are not necessary or when the focus is on generalities. In contrast, the adjective "visceroceptive" and the noun "visceroception" are encouraged to be used when referring specifically to visceral tissue origins, distinct from and not including somatic tissue origins.

Of course, it is crucial then that there is understanding of what viscera are, because, as is the case with the word interoception, there is more than one definition. We can recognize at least three types of definition: (1) one arbitrarily grouping certain anatomical structures under the label viscera, (2) another based on efferent innervation, and (3) a final one focusing on perceptual differences.

3.2.1 The dictionary definition

One definition used for distinguishing viscera from somatic tissue creates this divide as based on anatomical location, and is commonly found in dictionaries; it either labels (a) only the intestines, or either (b) all intra-thoracic, intra-abdominal and intra-pelvic organs as viscera (Berube et al., 2008). The problem with considering only the intestines, i.e. the part below the stomach as viscera, is that the stomach and organs located in the thorax can then neither be considered visceral, nor somatic - yet no other label is provided for these "gray zone" body tissues. As for the anatomy based definition which considers *all* intra-abdominal and intra-thoracic organs to be viscera, it simply classifies the remainder of the body as somatic tissue. That is: not only skin and skeletal muscles, but also joints and bones (Lewis, 1938). This is usually accompanied by a further arbitrary subdivision of somatic tissue distinguishing the skin from the remaining "deep" somatic tissue. Whether the circulatory system is visceral or somatic according to any such anatomical definition usually remains unmentioned, as the circulatory system branches out into all areas of the body, making it difficult to classify based on its location. Also, if viscera are strictly those organs located in the trunk, then the female reproductive system should be considered to be entirely visceral, whereas at least part of the male equivalent (in addition to the dermis) should be considered somatic. As no such claims are made by anyone, this implies that the anatomy based definition as given is not strictly adhered to even by its proponents, and that the dictionary definition is not sufficient by itself to classify tissues. For better communication, it is considered preferable to use definitions which do not leave any room for subjective interpretation and which do not require additional, implicit, unmentioned criteria.

3.2.2 Definition as based on efferent innervation

In contrast to the aforementioned dictionary definition, there exists a very straightforward, clear-cut physiology based definition that makes the distinction between visceral and somatic tissue as based on actual efferent innervation (Wolfsohn, 1914). Relying on existing knowledge of efferent somatic nervous system (SNS) innervation and autonomous nervous system (ANS) innervation to determine which tissues are respectively somatic and which ones visceral, deserves preference for two reasons.[4] First, it does not leave a single tissue of the entire body unmentioned, and would classify the circulatory system as visceral (Livingston, 1935). Second, it does not leave room for arbitrary individual

[4] Although the author of this dissertation recognizes that the Enteric Nervous System (ENS), has its own reflex activity independent from the ANS, both ANS and ENS innervated tissue are classified here under the label "visceral" as opposed to "somatic".

choices on which organic tissues to include under the label viscera, and which ones not, because physiologically verifiable, existent efferent innervation cannot be contested.

Note should be taken that making the distinction as based on efferent innervation differs on some important aspects from those who simply label all organs in the trunk as viscera and consider the remainder of the body as somatic. First, when basing ourselves on efferent innervation, we can determine that the skeletal system is in fact to be labeled as visceral, and not somatic (Kini & Nandeesh, 2012). One implication of this is that bone pain thus is to be considered a visceral, and not a somatic pain according to innervation. Another implication is that, in so far that sensory feedback from the skeletal system (including periosteum) contributes at all to proprioception, this would then be a visceral component contributing to the CNS representation of the body in space (proprioception), which is perfectly possible if we adhere to inclusive definitions of proprioception, interoception and exteroception, where all that matters is the phenomenological experience and not which type of receptors are involved in creating that experience.

Other differences between the dictionary definition and the efferent based definition for distinguishing somatic from visceral tissue relates to the classification of the skin, the esophagus, and the respiratory system. If we consult known information on efferent innervation, we can conclude the skin is in fact not a purely somatic tissue in contrast to what is often stated (Gibbins, 2013; Oaklander & Siegel, 2005). The skin actually contains both SNS as well as ANS innervation, making it a partially somatic, partially visceral organ. In psychophysiology this visceral aspect is well recognized, where dermal autonomous changes such as changes in pilo-erection and sweat secretion can be and are used to assess physiological aspects of emotion (Benedek & Kaernbach, 2011; Dawson, Schell, & Filion, 2007).

Like the skin, the esophagus is a single functional unit, yet its proximal section has SNS innervation, whereas the distal section has ANS innervation, also making the esophagus an organ which is partially somatic, and partially visceral. Unlike the skin, the esophagus has a clear division between the visceral and somatic parts. Also included in this list of mixed SNS and ANS innervation, although not strictly speaking an organ, is the respiratory system (Kuntz, 1944).

All three of the aforementioned – the skin, the esophagus, and the respiratory system – have a prominent role in the interoception literature and related research. Craig was the first to argue that some tactile sensations, such as sensual touch, are distinct from other touch sensations and are relayed to the brain together with other homeostatic sensations (Craig, 2002). While Craig based his argument on afferent innervation (see section 2.2.3), this chapter makes the distinction based on efferent innervation. The esophagus too is gaining increased attention in interoception research, as it allows to distinguish between viscerception (if stimulated distally, i.e. the lower part) and

somatoception (if stimulated proximally, i.e. the upper part) (Aziz et al., 2000). As for the respiratory system, since early human experience it has been the gateway to altering and gaining control over ANS function and thus control over the viscera (Sovik, 1999). In fact, one of the first written records where breathing is considered to be able to affect the viscera, is in a book by Tao Hongjing, written sometime around the end of the 5th or start of the 6th century, where six different methods of breathing are considered beneficial to the health and functioning of six different viscera (Zhang, Tao, Guo, Song, & Liu, 2007). Because respiration can be used to increase the inotropic output of heart rate (Shannahoff-Khalsa & Kennedy, 1993), which in turn increases heartbeat perception accuracy (Herbert, Herbert, et al., 2012), respiration may even be considered to be a gateway for altering heartbeat perception and perhaps also for altering other forms of viscerоception.

3.2.3 Definition as based on “typical” sensory properties

A final note on methods for distinguishing between somatic and visceral tissues concerns sensory properties. The viscera are often attributed three typical sensory properties that are thought to distinguish them from somatic tissue. These three visceral properties are said to be (1) the inability to volitionally bring visceral sensations into awareness, (2) poor discrimination of sensations, and (3) poor localization (Aziz et al., 2000; Dunckley et al., 2005; Dworkin, 2007; Ray & Neill, 1947). Each of these points will be addressed here.

As to the first point, although visceral sensations generally only enter awareness bottom-up (e.g., when homeostasis is disrupted, or in mental disorders), it is actually possible to volitionally attend in a top-down method to visceral sensations as is done in meditative practices. Even though such increased awareness does not necessarily imply increased accuracy (Ceunen, Van Diest, et al., 2013; Khalsa et al., 2008), nevertheless it is possible to volitionally attend to visceral sensations, which underscores that the first property associated with visceral sensations is not correct.

The second sensory property often associated with viscera, namely poor discrimination, i.e. poor perceptual accuracy, is indeed very common to most ANS innervated organs, yet not universally applicable to all. For example, there are subgroups of individuals who can very accurately perceive their heartbeat. One may argue such accurate heartbeat perception is possible due to heartbeats resonating in somatic muscle tissue overlying the heart region, thus implying there is not sufficient evidence for the existence of accurate viscerоception. However, even when sensations from overlying somatic tissues are absent, accurate heartbeat detection is still possible (Khalsa, Rudrauf, Feinstein, & Tranel, 2009), which indicates that good discrimination is possible even for at least one type of viscerоception, and perhaps also for other types.

As for poor localization, it is true that the majority of visceral sensations are phenomenologically experienced as vague, diffuse, or pertaining to a general area, rather than to a

precise spot. However, from an experience level, pain stemming from kidneys, appendix, genitalia, and anus all have known instances where these were subjectively experienced as sharp, and/or in a clearly localized way (Bajwa, Gupta, Warfield, & Steinman, 2001; Boyle, 1997; Fauconnier et al., 2013; Kafkia, Chamney, Drinkwater, Pegoraro, & Sedgewick, 2011; Sandella, Hartmann, Berkson, & Hong, 2012; Sejdinovic, Salihefendic, Pandza, & Zildzic, 2011), although none of these pains stem from tissues innervated by SNS efferents. Moreover, tactile sensations including itch, sensual touch and temperature can be fairly accurately localized. Taking into consideration that the skin is partly ANS innervated, this is yet another example which questions whether all visceral sensations are indeed poorly localized. Furthermore, note should be taken that not all somatic tissue sensations are characterized by accurate localization either (Feinstein, Langton, Jameson, & Schiller, 1954; Lewis, 1938).

3.2.4 Implications

One practical implication is that, when classifying body tissues as either somatic or visceral, it is suggested here *not* to combine classification as based on sensory properties with classification as based on efferents: this is simply not completely accurate and is confusing to the critical reader. An example of one such to be avoided, confusing statement would be: “we consider this organ as visceral BECAUSE it is ANS innervated and BECAUSE its sensations are poorly localized.” Instead, it would be better to say: “We consider this organ as visceral because it is ANS innervated. Sensations from *most*, but not all ANS innervated organs, including/excepted this one, are poorly localized.” When sensory properties are considered truly relevant to specific research conclusions or predictions, distinguishing viscera with sensory properties typical to most viscera, from other viscera with anomalous sensory properties may definitely have its value. E.g., one could make the distinction between those typical ANS innervated organs that are always poorly discriminated and/or poorly localized, as opposed to those few ANS innervated organs which can at times be accurately discriminated and/or accurately localized. *(Accurate discrimination refers to heartbeat detection through visceral afferents only; accurate localization, albeit not always, refers to kidneys, appendix, genitals, anus, and the tactile sensations - the latter being included because the skin is partly ANS innervated.)*

In practical, research oriented terms, this section clarifies how to classify stimuli as either visceral or somatic in such a way that if others apply the same method of classification, they will make exactly the same conclusions as to which body tissues are visceral and which are somatic. Moreover, this section also implies that stimulation of certain organs or systems can elicit sensations which are a combination of both visceral and somatic components. For example, respiratory stimuli such as loaded breathing (i.e., breathing against a resistance) and CO_2 inhalation have as visceral component the sensory feedback from the lungs and the CO_2 levels in the circulatory system, while the somatic

component is the sensory feedback from the respiratory muscles (Epstein, Manning, & Schwartzstein, 1995). Likewise, cold pain as induced by the cold pressor test (a test where subjects are required to submerge their hand in cold water), is not a purely somatic stimulus as is often suggested by those who interchange the terms “somatic”, “exteroceptive”, and “exogenous” as synonyms. Immerging the hand in cold water in fact affects visceral tissue in addition to somatic tissue. It does so through the baroreflex which involves the (visceral) circulatory system, but also because the skin is an organ with both visceral and somatic components, rather than being purely somatic. Moreover the cold penetrates beyond the skin, not only into the somatic muscle tissue beneath the skin, but potentially penetrating as deep as into the bones, for which there even is an English expression, namely “being cold/chilled to the bone”. Even without being literally cold to the bone, cold pain as induced by the cold pressor test has been known to have been subjectively perceived as radiating from the submerged hand all along the veins across the entire lower arm, as reported by participants in an earlier study of the author (Ceunen, Vlaeyen, & Van Diest, 2013). Such subjective experience of ‘spreading’ cold suggests that pain induced by the cold pressor is at least partly dependent on visceral sensory feedback stemming from the circulatory system, as described by Livingston (1935).

The most important implication of this section for research purposes, is that under natural circumstances visceral sensations are frequently accompanied by somatic sensations as is the case for heartbeats, and possibly also for ingestion of large amounts of food. Therefore it is imperative that before designing an experiment, the researcher determines whether it is absolutely crucial to elicit visceral sensations without eliciting any somatic sensations whatsoever, or whether ecological validity is more important. If the aim is merely to elicit a sensation that resembles a real life sensation as close as possible, it may not be necessary to come up with elaborate, time consuming or other effortful investments intended to annihilate any possible somatic sensation from co-occurring with a visceral sensation. Moreover, these contraptions intended to block out somatic sensations can create other (albeit constant) variables into an experimental set-up, affecting the outcomes of a study just as much, but merely in a different way than when such ‘precautions’ would not have been taken.

3.2.5 Overview of section 3.2

In summary, this section argues in favor of labeling visceral and somatic sensations specifically when needed, rather than invariably lumping them under the more generalized terms interoception and exteroception, and it also argues that interoception and exteroception are not synonyms for respectively visceral and somatic. Although it is true that sensations arising from viscera are most often contributing to the phenomenological interoceptive percept, in certain instances visceral sensations can potentially contribute to the phenomenological exteroceptive percept of what is going on in the environment around us. It was further argued that viscera are preferentially to be defined

as those organs with efferent ANS innervation, while somatic tissues are to be defined as body tissues with efferent SNS innervation. It has been brought to the reader's attention that some organs such as skin and esophagus, or functional units such as the respiratory system have a combination of both types of innervation. Furthermore, although poor discrimination and poor localization are common to most ANS innervated organs, it is inaccurate to conclude that an organ must be somatic simply because sensations thereof can be accurately perceived and/or well localized. In other words, it is incorrect to state that poor localization and poor discrimination are a *universally* defining characteristic that allow to determine whether an organ is to be considered ANS or SNS innervated. Using efferent innervation to guide our definition allows for physiologically based conformity across researchers in determining which kind of sensations are to be considered visceral, which ones somatic, and which ones a combination of both. Also of note is that in designing experiments where ecological validity is the aim, attempts at creating 'purely' visceral sensations may not even be necessary, although these may be informative.

3.3 The homeostatic afferent pathways and early CNS processing of homeostasis

When Bud Craig redefined interoception as the sense of physiological status of all tissues of the body, he specified this "sense" as being a CNS representation, while at the same time arguing that this representation starts at the receptor site and is relayed via what he labeled as the homeostatic pathway (Craig, 2002). While Craig labels both the relay of the homeostatic state of the body (from receptor site up to primary levels of processing) and higher order levels of processing as interoceptive, we prefer to use two different labels to distinguish these two aspects. Specifically, we would reserve interoception to refer to the higher order processing, which occurs once the mid-insula gets involved (see also section 3.4). The process from receptor site up to primary areas of processing are labeled here as homeostatic pathways (section 3.3). Take note that there is not just one homeostatic pathway, but instead there are at least three to five (Critchley & Harrison, 2013) depending on how one counts, as will be outlined in the paragraphs immediately hereafter.

The spinal homeostatic pathway refers to all processes as illustrated in figure 1, prior to mid insula involvement (Craig, 2010). It starts with stimulation of Aδ and/or C-fiber receptors, is relayed via the first (and also the second and fifth) lamina of the dorsal horn of the spinal cord, on to the brainstem homeostatic regions which form a pre-cortical homeostatic representation, and then to the posterior, basal and medial thalamic nuclei. Finally there is activation of the primary sensory processing area for homeostatic sensory input, namely the dorsal posterior insula. This spinal homeostatic pathway is distinct from the spinal relay of non-homeostatic sensory information, which

is schematically depicted on the right hand side of figure 1, and which for the purpose of this thesis needs not be discussed in further detail.

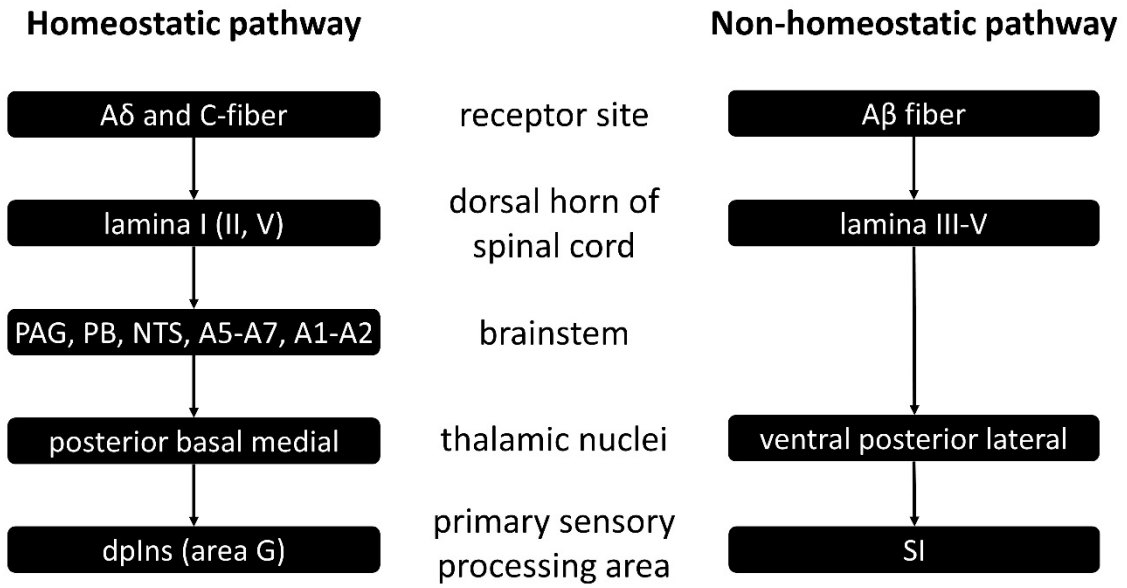

Figure 1. Schematic representation of *spinal* homeostatic and non-homeostatic afferent pathways as based on (Craig, 2010). (PAG = periaqueductal gray; PB = parabrachial nucleus; NTS = nucleus tractus solitarii a.k.a. nucleus of the solitary tract; dpIns = dorsal posterior insula; SI = primary somatosensory cortex)

Other than (1) the *spinal* homeostatic pathway, there are also other routes via which primary homeostatic processing can occur (Critchley & Harrison, 2013). There is the (2) *cranial* homeostatic pathway. It is that of the cranial nerves, such as vagus and glossopharyngeal nerves carrying information from the receptor sites to the brainstem – first to the nucleus of the solitary tract (NTS), and then on to the parabrachial (PB) nucleus (PB) and periaqueductal gray matter (PAG) – and from there on to thalamus, hypothalamus, amygdala, and ultimately to the anterior cingulate cortex (ACC) and the insula. It may be of interest to note here that taste – often categorized as one of the distinctly non-interoceptive sensations – is in fact relayed via cranial nerve afferents (Craig, 2005).

Then there is also the (3) *humoral* homeostatic pathway, which reaches the CNS via circulating substances. The humoral pathway refers in fact to at least three different pathways of information transduction, which all share the commonality that they are in first instance activated via circulating substances. The (a) *ventricular* (or classical) humoral pathway detects changes in substances present in the third and fourth ventricles, and first engages the circumventricular organs which are located adjacent to these ventricles; these include the area postrema (AP), the organum vasculosum of lamina

terminalea (OVLT), and the subfornical organ (SFO). The humoral info processed here in turn projects to the NTS, the hypothalamus, the PB, sympathetic medullary nuclei, the dorsal motor nucleus, the nucleus ambiguous, midline thalamic nuclei, and again the insula and ACC. The (b) *blood-brain* (or nonclassical) humoral pathway is that which detects changes in those substances passing the blood brain barrier. It involves the NTS, hypothalamic nuclei, the medial amygdala nucleus, and monoamine systems, and can influence the information relay between ventral striatum, insula, and cingulate. The (c) *microglial* (or extraneuronal) humoral pathway is that in which the microglia in the circumventricular organs, leptomeninges and choroid plexus respond to peripheral presence of pathogens and inflammation. The changes taking place in these microglia in response to these signs of infection and tissue damage activate a cascade of microglial activation across the CNS.

3.4 Interoception as integrated percept

Although the spinal, cranial, and humoral homeostatic pathways are the most direct routes of sensory feedback concerning the status of all tissues and the state of the body, they are not exclusive contributors to the highest order percept of the body status. In constructing a central, higher order representation of the body status (i.e. interoception) the CNS relies on all available information, which it integrates in the mid insula (Craig, 2008). Other than homeostatic feedback relayed from the dorsal posterior insula (or primary interoceptive cortex) to the mid insula, the mid insula also receives input from the secondary somatosensory cortex, thus effectively allowing for the integration of spinal non-homeostatic afferent information (see figure 1) in the interoceptive percept. In addition, also visual, auditory and vestibular feedback are integrated at the mid insula (Craig, 2008). The mid insula further communicates with and integrates information from the amygdala regarding stimulus salience and emotional memories, as well as with the hypothalamus regarding current state of the ANS and of ongoing metabolic processes (see Fig. 2, adapted from (Craig, 2008)). Thus, the mid insula is considered to be the locus responsible for the integrated re-representation, feature extraction and cross-modality integration, i.e., the core structure needed for what can be considered interoception (Craig, 2010). When this integrated re-representation is relayed to the right anterior insula where also subjective time perception is processed, interoception enters the realm of apperception, i.e. conscious interoception. As can be seen in figure 2 from the presence of reciprocal connections represented by two-way arrows, as well as is indirectly evident from section 2.2.3, arriving at this higher order integrated percept involving the mid insula and the anterior insula, is in fact not an entirely sequential hierarchical process (Critchley & Harrison, 2013). Although there is a posterior-to-mid-to-anterior processing in the insula, there is also a lot of cross-talk between many of the “lower” areas with one another as well as cross-talk from these areas from and to the mid insula and the anterior insula. As such, interoception is in fact the sum total of all structures involved in addition to activation of mid

and anterior insula – it is the product of a neural matrix for body state perception (Craig, 2005; Critchley & Harrison, 2013; Legrain et al., 2011; Moseley et al., 2012).

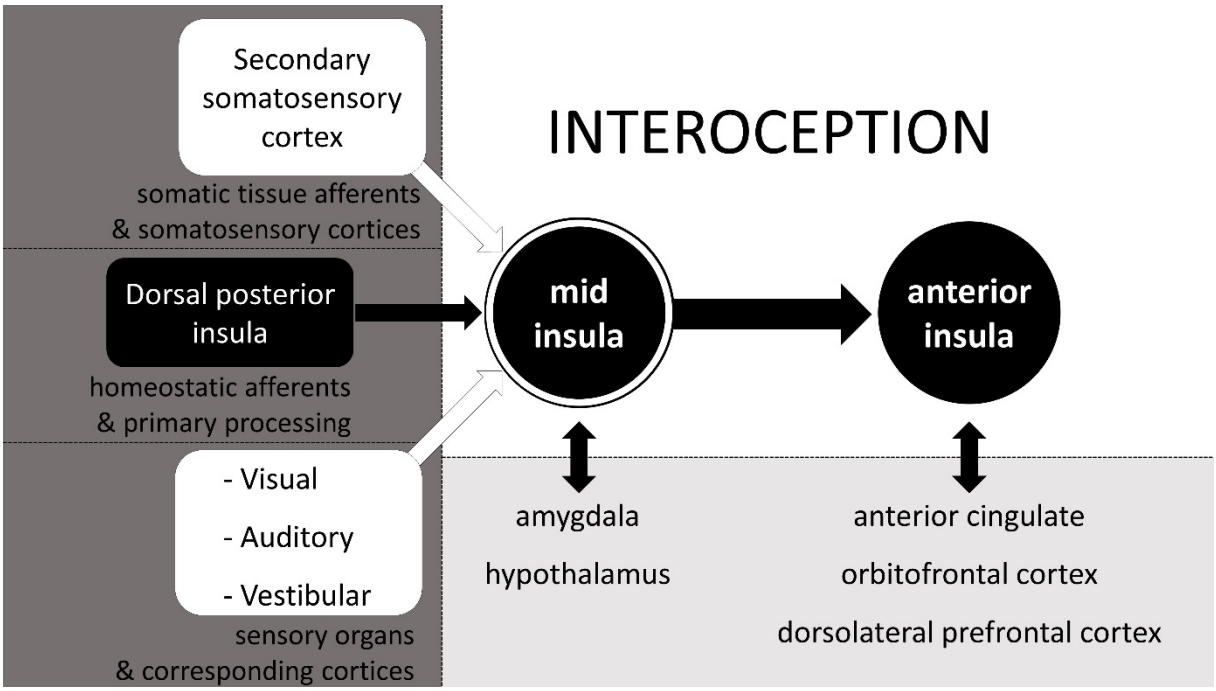

Figure 2. The neurophysiology of interoception. Schematic simplification of the neurophysiology behind the cross-modal integrated (re)representation of the body status, otherwise known as interoception. Adapted from Craig (2008)

Examples from the subjective perception of body state will be given here and in paragraphs immediately following this one, to illustrate the part that each of the sensory inputs as illustrated in figure 2, contribute to interoception. In figure 2, we can see that somatic sensations relayed via the secondary somatosensory cortex can potentially contribute to interoception. Although the ability to accurately perceive heartbeats does not necessarily require afferent feedback from somatic tissue (Khalsa et al., 2009), in cases were such afferents are involved, there is activation of somatosensory areas, which results from sensations relayed via the spinal non-homeostatic pathway (see figure 1). It is likely that such clear somatic sensations are most common with inotropic activation of the heart, as manipulations that increase inotropic output, also increase cardioceptive accuracy (Herbert, Herbert, et al., 2012).

As seen in figure 2, vision can play a significant role in interoception. This contrasts to popular views, wherein vision is generally considered to be a sensory faculty which solely contributes to the perception of the surrounding environment. An example where a visual experience is part of interoception is the gray-out that often occurs shortly before the onset of syncope. For the individual, subjectively this gray-out is part of the cascade of sensations of the fainting experience and of the

percept that the homeostatic status of the body is disrupted (Kamiya et al., 2005; Shukla & Zimetbaum, 2006). Another well accepted finding that supports and illustrates the notion that vision can in fact contribute to the perception of the body status comes from satiety research, in which visual feedback has long been recognized as one of the factors affecting food intake, a basic homeostatic function crucial for survival (Berthoud, 2006; Cornier, Von Kaenel, Bessesen, & Tregellas, 2007; Morton, Cummings, Baskin, Barsh, & Schwartz, 2006). Moreover, visual feedback also affects the experience of acute pain and phantom limb pain (Chan et al., 2007; Mancini, Longo, Kammers, & Haggard, 2011; Ramachandran & Rogers-Ramachandran, 1996), which further underscores that vision has the potential to contribute to the perception of the own body state. Even the sight of facial expressions of others can affect processing of information about the own body state - at least for pain (Khatibi, Vachon-Presseau, Schrooten, Vlaeyen, & Rainville, 2014; Mailhot, Vachon-Presseau, Jackson, & Rainville, 2012; Wieser, Gerdes, Reicherts, & Pauli, 2014).

Not only vision has the potential to contribute to interoception. Auditory information or the disruption or absence thereof can just as much contribute to the phenomenological interoceptive percept. The most obvious example is tinnitus, which can indicate either the onset of syncope, inner ear damage, or which can be part of a set of symptoms which indicate some sort of homeostatic disruption (Bitterman, 2004; König, Schaette, Kempter, & Gross, 2006; McGuinness & Harris, 1961; Nam, Lewis, Nakajima, Merchant, & Levine, 2010; Shukla & Zimetbaum, 2006). Tinnitus also includes instances of actually hearing one's own heartbeat, which is referred to as pulsatile tinnitus and which can go together with high blood pressure or other circulatory abnormalities (Mattox & Hudgins, 2008). Of course not all forms of pulsatile tinnitus correspond to the heart-rate, e.g. when pulsing is caused by spasms of ear muscles, but even then the pulsatile tinnitus reflects a physiological abnormality, i.e., deviation from the homeostatic state of the body. Moreover, verbal information can also alter perception of the body state and affect it, for example under social stress or during hypnosis (Darwin, 1872; Drummond et al., 2003; Tan, Hammond, & Gurrala, 2005). All these are mere examples which indicate auditory feedback can and does at times contribute to the perception of the body state.

As for the potential of vestibular sensory information adding to interoception, many may have experienced it as the feeling of dizziness or vertigo which sometimes accompanies physical illness and therefore can be indicative of it. From all these examples, it should be clear that really any type of sensory information, and not merely that from homeostatic pathways can get integrated into the overall body percept. It is only because of this integration of sensory input that biofeedback is possible in the first place and can help in the treatment of many psychiatric disorders in which interoception plays a major role (Schoenberg & David, 2014). Of course the selection of examples listed here are by

no means exhaustive of how non-homeostatic sensory information can contribute to interoception: they merely serve as illustrations of how multisensory interoception truly is.

Note also that interoception is defined as a *cross-modal* integrated representation of the body status, rather than merely a *multisensory* representation. It is cross-modal because this phenomenological experience of the body status not only integrates input from a variety of peripheral sensory channels, but also integrates information from, and cross-talks with different structures within the CNS as can be seen in figure 2. Much of the information relayed via the sense organs which gets integrated in the interoceptive percept, can only be integrated because learning enabled the individual to link these percepts as informative on the body state. This learned integration can be effected via conditioning or other forms of learning (e.g., Bevins & Besheer, 2014; Ceunen, Zaman, et al., in preparation; Pappens et al., 2013). Anil Seth (2013) proposes that interoception, or interoceptive inference as he labels it, is not just passive, bottom-up processing, but is something which also involves active top-down activation to make predictions of the causes of sensory input. His view is based on the central idea of predictive coding, which is that perception is a process of not only afferent feedback, but also of predictions, and ultimately the integration of both, resulting in prediction errors. Predictive coding models also consider MUS as arising from not only peripheral sensory feedback, but also from prior beliefs, where attention, attributed agency, expectation, prior experience and even cultural beliefs all play a role in perception of symptoms (Edwards, Adams, Brown, Pareés, & Friston, 2012). This is clear for example from the effect of instruction in decreasing (placebo) or increasing (nocebo) visceral pain intensity (Schmid et al., 2013). More support for the argument made here, is that interoception can be manipulated by something as simple as categorizing interoceptive sensations versus rating those same sensations on a continuous dimension (Petersen, Schroijen, Mölders, Zenker, & Van den Bergh, 2014). The change in interoception with this sort of experimental manipulation is likely effected via a mechanism of biasing perceptual decision making, as shown extensively in categorization research involving exteroception. Further supporting the argument of CNS involvement in body state perception are corollary discharge models of effort, which hold that the perception of physical exertion is entirely centrally generated, rather than resultant from somatic afferents (Marcora, 2009). Taken together, we should at least consider the possibility that body state perception other than perception of effort may also be in part centrally generated.

It has been suggested by Paulus and Stein (2010) that with increased ambiguous or noisy sensory input from the homeostatic pathways and decreased accuracy of perception, the brain relies especially on itself (as well as on alternative sensory channels) enhancing top-down modulation and creating a self-referential biased percept of what is going on with the body. Whether individuals with somatoform disorders, mood disorders and anxiety disorders are less or more viscerоceptively

accurate is hard to conclude given opposing research findings. That people with these disorders are not entirely homogenous with regard to their ability for heartbeat detection, can be related to findings from McGrath and colleagues (2013). Their findings suggest that at least for major depression, and perhaps for mood and other disorders, there are two subgroups of patients: one group consists of individuals with an overactive anterior insula, and the other of individuals with an underactive anterior insula. These two distinct neurological biomarker patterns of these two subgroups are suggestive respectively of accurate and inaccurate perceivers. This would explain why findings regarding cardioceptive accuracy in the aforementioned disorders are contradictory. Regardless of the accuracy with which individuals with mood and anxiety disorders can perceive sensory homeostatic afferent feedback, individuals with such disorders excessively rely on sources other than actual bottom-up homeostatic pathways, giving more weight to maladaptive cognitive-emotional schemes of interpretation (Paulus & Stein, 2010). All of the aforementioned further contributes to the view that the perception of the body state, i.e. interoception, is a truly multimodal percept.

4. Conclusions

Although Sherrington (1906) originally came up with and used the label interoceptive as a synonym for things visceral, over the course of time, interoception has come to mean much more than just that. While interoception is sometimes referred to as viscerosensory integration (Critchley & Harrison, 2013), interoception is more than the central sensory integration of afferents stemming from only the viscera. Interoception has in fact come to refer to a multimodal integration not restricting itself to any sensory channel, not restricting itself to mere sensations, but also relying on learned associations, memories, and emotions and integrating these in the total experience which is the subjective representation of the body state. Interoception defined as such includes any form of pain, not just visceral pain, but somatic pain as well.

This inclusive definition of interoception is not new. Rather, this chapter expands on this view and the formerly made association with the inclusion of pain in this definition, and is guided by the most commonly accepted definition of pain to serve as inspiration for the definition of interoception. Pain is defined as based on its phenomenological experience rather than referring to the physical origin of the pain sensation or any physiologically objectively quantifiable aspect (H. E. Merskey & N. Bogduk, 1994). The IASP considers pain to be a psychological state, and although it recognizes that pain often has a proximate physical cause such as a noxious agent activating nociceptors and nociceptive pathways, it emphasizes that this need not always be the case, and that therefore the presence or absence of a noxious stimulus is not relevant in determining whether there is pain or not, and neither is the activation of nociceptors or nociceptive pathways.

Like this definition of pain then, so too has "interoception" become such a broad concept that this chapter argues it should be defined as a subjective experience of the body state. Although in many instances, this experience may well be elicited by a peripheral change in homeostasis, this need not necessarily always be so. Independent of the phenomenological experience which is interoception, aspects potentially contributing to interoception can be classified in myriad ways. One way is to consider whether sensations have an endogenous or exogenous origin. Whatever the actual source of a sensation, endogenous or exogenous, it does not determine whether a perceived sensation is to be considered interoceptive or not. The only thing determining whether something is interoceptive is whether it contributes to the subjective perception of body state. The same can be said of the distinction between somatic tissue and viscera. Although it is often relevant to distinguish visceral from somatic tissue, it does not mean sensations stemming from somatic tissue cannot contribute to the phenomenological percept of the status of the body. To avoid confusion between visceral sensations on the one hand, and the subjective feeling state that is interoception on the other, it is suggested in this chapter to not use these two related but distinct concepts as synonyms. In particular, it is preferable to keep words which contain a direct linguistic reference to viscera (e.g. viscerosensory, visceroceptive, visceroceptor, visceroception) reserved for instances where the distinction between ANS/ENS efferent innervated tissue on the one hand and SNS innervated tissue on the other is of equal or more relevance than the sum phenomenological experience of the general state of the body.

Homeostatic pathways (including early CNS homeostatic processing) have also been discussed in this review, and are considered to provide the most direct sensory feedback on the state of the body. The author prefers to label these as *homeostatic* rather than interoceptive pathways, and only speaks of interoception from the point in processing onward where there is a higher order integration of information, sensory and neural, taking place to form a body state representation in the CNS. Thus, the "-ception" in "interoception" is taken to no longer refer to "reception" (i.e., receiving) of stimulation, but rather the CNS "perception" of the body state. Perception itself is always an inherently flawed and subjective reconstruction of reality by the CNS, never a one on one accurate representation. Hence, the core of the definition of interoception is on the subjective experience above all else; thus we can say the brain is the true source, i.e. the real origin of interoception.

CHAPTER 2

Interoceptive fear and its measurement

Interoceptive fear – i.e. apprehension of bodily sensations (Shear et al., 1997) – has been suggested to be adaptive in helping prevent or restore disruption of homeostasis (Ceunen, Vlaeyen, et al., 2013) which can be achieved through behavioral action or inaction (Bolles & Fanselow, 1980). Interoceptive fear has also been hypothesized to play a role in a number of psychiatric and somatoform disorders. Somatoform disorders are syndromes without an identifiable organic or other physical abnormality, in which cognitive and/or emotional components are thought to play a major role. In psychiatric and somatoform disorders, typical feared bodily sensations include fear of dyspnea, arousal, and tachycardia as in panic disorder (PD) (Acheson, Forsyth, Prenoveau, & Bouton, 2007), fear of sensations along the digestive tract as in Funtional GastroIntestinal Disorders (FGID) (Labus et al., 2004), and fear of pain as in pain disorders such as fibromyalgia (Mccracken, Zayfert, & Gross, 1992). Fear of such bodily sensations is not limited to these three types of disorders, but likely extends to a variety of mood and anxiety disorders (Paulus & Stein, 2010), Chronic Obstructive Pulmonary Disease (COPD; Moore & Zebb, 2000), Medically Unexplained Dyspnea (MUD; Han et al., 2008) as well as other Medically Unexplained *Symptoms* (MUS; Deary, Chalder, & Sharpe, 2007).

Interoceptive fear can arise and/or can be maintained through fear conditioning (Acheson et al., 2007; Bouton, Mineka, & Barlow, 2001; Ceunen, Zaman, et al., in preparation; De Peuter, Van Diest, Vansteenwegen, Van den Bergh, & Vlaeyen, 2011; Mayer, 2000; Meulders, Vansteenwegen, & Vlaeyen, 2011; Pappens et al., 2013; Zaman et al., submitted). In interoceptive fear conditioning, an originally benign sensation can come to elicit a fear response, due to past associations with an unpleasant outcome. Indeed, many somatoform disorders seem to have been preceded by a history of a single or repeated actual tissue damage such as tissue inflammation (Cervero & Laird, 1999). Moreover, this indicates that the occurrence and relevance of interoceptive fear is likely not restricted to disorders without an identifiable physical abnormality, but may also occur in response to accurate interoceptive perception of actual homeostatic disruption.

Although interoceptive fear conditioning has a strong pedigree in the understanding of the aforementioned disorders, relatively little research has elaborated on the basic fear response topography to interoceptive stimulations used in the laboratory. The most likely reason for the scarcity of interoceptive stimulation in fear research is that interoceptive stimuli are more difficult to control experimentally: onset and offset are not always as discrete as with the presentation of more

commonly used aversive stimuli such as unpleasant photos, videos, odors, or sounds, which are generally considered exteroceptive. Moreover, administration of interoceptive stimuli is usually accompanied by a number of practical constraints, whereas the administration of exteroceptive stimuli is relatively easy. Another factor at play which has probably disfavored the use of interoceptive stimuli, is that these stimuli often do not merely elicit fear responses, but also elicit physiological regulatory responses (responses aimed at restoring homeostasis), making it difficult to interpret the elicited responses. A consequence of the almost exclusive use of exteroceptive stimuli in fear research, is that it remains unclear whether the existing knowledge base on fear and fear responding is at all applicable to interoceptive fear.

The limited studies on interoceptive fear are the cause for our limited knowledge, and general lack of theories in this area. The objective of this project is to investigate and describe fear responding to different kinds of aversive interoceptive stimuli which can be administered in a quantifiable way (see chapter 3): this will allow to describe the fear response topography to these various interoceptive stimuli and to explore possible unique as well as common qualities of each, relative to each other and relative to more commonly used exteroceptive stimuli. A basic understanding of the fear response topography to interoceptive stimuli in its turn will facilitate the interpretation of results of research on the acquisition and treatment of interoceptive fears.

Interoceptive stimuli

Although the selection of interoceptive stimuli for usage in research is undeniably influenced by pragmatic limitations, selection of stimuli starts or should start from a conceptualization of interoception, as for this dissertation has been done in chapter one. Such a conceptualization serves to provide a solid argumentation for the inclusion of the stimuli that are used, an argumentation for why the stimuli used can be considered interoceptive, and how they compare to one another.

To recapitulate: the working definition of interoception adopted in this thesis is an inclusive one, it is the perception of the state of the body – an ability that aids in maintaining homeostasis (Craig, 2002) and thus is crucial to ensure proper functioning of both body and brain. Defined as such, any perception of disrupted homeostasis, regardless of whether it is accurate or not, is considered interoception. This also includes any form of pain, either somatic (efferent SNS innervation) or visceral (efferent ANS innervation). The interoceptive sensations selected for this project were cold pain as induced by the cold pressor test (CPT), hypercapnia induced with inhalation of CO_2 enriched air, increased breathing effort and dyspnea as elicited by breathing against a resistive load, and esophageal sensations induced by means of an esophageal balloon which could be distended. Each of

these stimuli will be addressed in separate paragraphs below, and we will argue why we consider these stimuli interoceptive and to what degree they share similarities.

Cold pain as induced with the CPT requires research participants to submerge their hand in painfully cold water. Cold and cold pain is perceived via Transient Receptor Potention (TRP) ion channels TRPM8 and TRPA1, located in nerve endings in the skin and joints (Descoeur et al., 2011; Story, 2006). Moreover, saline injections of different temperatures indicate there are also thermal receptors in the vascular system which are responsible for feeling cold and cold pain (Fruhstorfer & Lindblom, 1983). Although acute pain can often be attributed to an external stimulus, we argue that the *existence of the stimulus* (in this case, cold water) in the immediate environment does not take away the perception of the personal space, i.e. that *the body is affected* by the stimulus (interoception) (Legrain, 2011). Cold pain induced by the CPT is *subjectively* experienced in local skin (partly visceral, partly somatic), muscles (somatic), and sometimes even joints and bones (visceral) – the latter being clear from the expression "feeling cold to the bone". CPT induced cold pain is fluctuating and can radiate to surrounding tissues, much like the sensation of cold saline injections radiates along the blood flow from the point of injection onwards. This radiating of cold, reduced skin blood flow, and elicitation of the baroreflex indicate the CPT affects the cardiovascular system (Shibahara et al., 1996). The involvement of the cardiovascular system illustrates: (1) that the CPT induces a genuine homeostatic disruption, and (2) that sensations stemming from the CPT have a strong visceral component, making the CPT at least partly interoceptive even for those who adhere to less inclusive definitions of interoception than we (see chapter 1). The cardiovascular system itself is involved in a multitude of visceral sensations: heart beat perception, indirect perception of changes in blood pressure (for example through pulsatile tinnitus, headaches or other), sensations of localized or general cold (in response to cold, to affective stress, or as in Raynaud's syndrome), or warmth (such as blushing and hot flashes). Due to the cardiovascular system's intricate link with the respiratory system in gas-exchange, stimulation of the cardiovascular system can induce alteration of breathing, which adds another interoceptive component to the CPT.

As respiratory stimuli, increased CO_2 levels and respiratory loading will be used. Although both induce dyspneic sensations, they stimulate distinctive systems and as such create different sensations. Apart from inducing air hunger, inhaling increased levels of CO_2 alters the respiratory behavior, and may be accompanied by feelings of dizziness, trembling, faintness, palpitations, sweating, feelings of unreality, cold, and/or an uncomfortable feeling at the chest, and sometimes paresthesia (Vandenhout & Griez, 1984). These symptoms resemble the interoceptive sensations accompanying a panic attack. Effects of CO_2 inhalation are thought to be cumulative, with less effect on the first few breaths. Most of the sensations following CO_2 inhalation are due to hypercapnia, but a taste in the

mouth (Chandrashekar et al., 2009) and detection through trigeminal afferents (Thurauf, Gunther, Pauli, & Kobal, 2002) is specific to actual *inhalation* of CO_2. Intuitively one could presume that a taste in the absence of a solid or liquid in the mouth leads to a reappraisal of the taste sensation as physical symptom. Of course it could be contested that the taste sensation following CO_2 inhalation is appraised as resulting from the mouthpiece or inhalation of gas bearing no link to the disruption of homeostasis evident from the other sensations. However, the attribution of the body state sensations to an external agent does not take away the sensations still are informative about what is happening to the body.

As to loaded breathing, there exist four types of respiratory load (Younes, 1995). In this project we have applied only one type, namely flow-dependent resistive loads. Resistive loads require extra effort from the respiratory muscles – the diaphragm and intercostals- during breathing, in order to maintain flow rate and volume. The sensation is comparable to breathing through a narrow tube or a (drinking) straw. Using this analogy, we can state that a higher load compares to a lower load as breathing through a very narrow straw compares to breathing through a straw with a larger diameter. Loads can be administered solely at inspiration, solely at expiration, or both at inspiration and expiration. Unlike CO_2 administration, loaded breathing can be noticed from the first breath if the load is above threshold level. Nevertheless, prolonged loaded breathing may also have some cumulative effects, as respiratory muscles can become fatigued. Although administered through a mouth piece, sensations induced by loading are interoceptive in that they are informative about the state of the body. Sensations induced by loaded breathing resemble dyspneic sensations experienced in COPD, asthma and other types of obstructed breathing.

For the gastro-intestinal stimulation balloon distensions in the esophagus are used, with stimulation intensity (pressures) individually adjusted. Although for the other interoceptive stimuli fixed intensities were used, an exception was made for esophageal stimulation. This is because perceived intensity is presumed to be partly dependent on individual differences in esophageal diameter and tissue compliance. Also of importance is the invasiveness of the procedure and the perception of control: for safe removal of the esophageal probe, participants preferably should rely upon the experimenter (whereas with respiratory stimuli subjects are self-capable of interrupting stimulation). It was reasoned that individually adjusted discomfort thresholds for esophageal stimulation may alleviate some of the context-related stress and decreases the likelihood that participants wish to terminate their participation prematurely. The esophagus is interesting for its clearly distinct innervations, with the proximal esophagus being somatic and the distal visceral. This allows not only to observe the response topography to aversive gastro-intestinal sensations, but leaves open the opportunity to understand the relative contribution of innervations on this response

topography. Although both somatic and visceral sites provide interoceptive feedback, the sensations are clearly distinct: sensations at the upper esophagus are clearly localized, whereas sensations of the lower esophagus are more spatially (Aziz et al., 2000) and even temporally diffuse – temporally diffuse in the sense that sensations last beyond stimulus termination (Strigo, Bushnell, Boivin, & Duncan, 2002).

All of the above stimuli were chosen to emulate sensations relevant for debilitating disorders that are currently poorly understood and for which interoceptive fear (conditioning) has been hypothesized as a potentially contributing mechanism (Acheson et al., 2007; Bouton et al., 2001; De Peuter, Van Diest, Vansteenwegen, Van den Bergh, & Vlaeyen, 2011; Mayer, 2000). In chronic pain disorders, some patients display fear of pain (De Peuter et al., 2011). Patients with panic disorder typically fear sensations that resemble those evoked by CO_2 (Acheson et al., 2007; Bouton et al., 2001). Patients with COPD or asthma and comorbid panic disorder typically fear difficulty with breathing. Patients with IBS suffer from gastro-intestinal sensations and typically anticipate and fear those sensations (Mayer, 2000). The assumption that such fears can be acquired, is in line with findings from clinical studies that they can also be extinguished with cognitive-behavioral procedures, (Craske et al., 2011; de Jong et al., 2005), although clinical implications for treatment are beyond the scope of the project described in this dissertation. The current project intends to provide a base of knowledge on the physiological and subjective response topography in response to a variety of aversive interoceptive sensations. The project is explorative in nature as systematic research on this topic is hard to find. This project can then serve future research more specifically aimed at generating a knowledge base for fundamental research on interoceptive fear (learning). Given that homeostasis is governed by specific sensations calling for specific behavioral responses –e.g., hunger prioritizing food intake, thirst water intake, dyspnea air intake, and pain withdrawal from or avoidance of the source of pain – we have included a variety of stimuli to account for and observe any unique qualities each of these may have. For clinical application, any specificity of responses may imply that customized approaches or measures could be necessary for studying or treating different interoceptive fears.

Fear and unpleasantness

Several models of human fear responding distinguish between different stages of fear-responding. Sokolov (1963) proposed that different patterns of autonomic responding reflect different modes of stimulus processing. He identified the orienting response as a response characterized by fear bradycardia (decelerating heart rate), which can be indicative of information intake (Graham & Clifton, 1966; Lacey, 1958). In contrast, the defensive response (DR) is characterized by hear rate acceleration, reflecting a decrease of information intake and an increase in action tendencies.

Blanchard and Blanchard (1989) have pointed to parallels between lower mammalian species and humans regarding primitive, self-preservation behaviors such as defensive responding. Based on animal models, these researchers conclude that there is a distinction between anxiety, fear, and defensive action, based on differences in threat value. Anxiety is particularly present in situations of a not-yet-identified, potential danger, and is characterized by risk assessment behavior and generalized vigilance. Fear sometimes follows anxiety, and is only present once a specific threat (e.g., a predator) is detected; it is characterized by freezing and selective attention, and is mediated by activation of prefrontal cortices such as the rostral anterior cingulated cortex (ACC) responsible for response selection and pavlovian fear learning, and medial Orbitofrontal cortex (mObfc) for assessing threat value (Mobbs et al., 2007; Tang et al., 2005). In the final stage when the predator is approaching, defensive action (and fear induced analgesia) occurs. Defensive action is mediated by activation of phylogenetically older midbrain structures such as the periaqueductal gray (PAG).

These three distinctions are also made in the defense cascade model by Lang and colleagues (1997). It subdivides human defensive fear responding into three successive (i.e., cascading) but distinctive phases, which have phase-specific patterns of peripheral psychophysiological responding. In this model, anxiety, fear, and defensive action are three consecutive phases respectively renamed 'pre-encounter', 'post-encounter' and 'circastrike'. A more recent fear response model by Schauer and Elbert (2010) further identifies three additional phases related to the onset of dissociative shut-down, such as fright induced catatonia, fainting, and the transition phase from the former to the latter.

Thus, it follows from stage-models of defensive responding that fear-related responses then, are not a simple, unidimensional, homogenous construct. This complicates the assessment and study of fear – also in the current project. For example, when asked to self-report perceived levels of one's own fear, how do we know what people are actually rating? Do they perceive the different phases of fear as single entity, and the different phases merely as different intensities of fear? I.e., would only the last phase of fear (fainting) be rated highest on fear intensity, and would the earliest phases such as pre-encounter, post-encounter and circastrike respectively be rated from low to only medium levels of fear? Different individuals may also have different inclinations as how to rate their own fear. In addition to the heterogeneity of the concept 'fear' affecting self-reported fear ratings, ratings may further be affected by response bias.

Obviously then, low levels of self-reported fear do not necessarily indicate that the paradigms used are irrelevant to the study of fear. However, it does indicate that mere self-report of fear is not sufficient to study interoceptive fear. One self-report measure that may aid in the study of fear is expectancy. Expectancy is a measure often used in studies on human fear conditioning. It refers to the likelihood with which someone expects an aversive event to occur (Boddez et al., 2013).

Biphasic accounts of emotion can give a further indication which additional measures could be useful to study fear. These biphasic accounts of emotion categorize emotions based on two aspects: motivational direction and motivational intensity (Bradley & Lang, 2007). Motivational direction refers to whether an emotion is 'positive' (approach) or 'negative' (avoidance), while motivational intensity refers to the level of arousal. From this perspective then, all phases of fear fall into the negative category, and have at least somewhat higher arousal than everyday baseline levels of arousal. Therefore it makes sense for the studies described in this dissertation to include both measures of motivational direction and measures of arousal.

For arousal there exists a 9-point language-free Self-Assessment Manikin (SAM-Scale, Bradley & Lang, 1994). This is validated scale composed of nine line-drawings assembled in ascending order from an image representing very low arousal on one end of the scale, and extremely high arousal on the other end of the scale. In addition to this simple self-report measure, arousal can also be assessed with psychophysiological measures. Quite commonly used to assess sympathetic and emotional arousal is skin conductance (Dawson et al., 2007; Wallin, 1981). Skin conductance directly reflects activity of the eccrine sweat glands, even before secretion of sweat. As these glands are innervated by the sympathetic chain of the autonomous nervous system, skin conductance reflects sympathetic arousal as controlled by the hypothalamus. Because of excitatory hypothalamic and amygdalar control, and inhibitory hippocampal control, skin conductance is also a measure of emotional arousal (Sequeira & Roy, 1993). Furthermore, skin conductance can indicate aspects of attention, such as stimulus novelty, intensity and significance which are related to activity in the ventromedial prefrontal cortex, right inferior parietal region and anterior cingulate (Dawson et al., 2007).

The SAM-scale described in the previous paragraph also has an item to assess the motivational direction experienced. This item ranges from unpleasant (as depicted by a manikin with a sad expression) on the one end, to pleasant (a manikin with a smile) on the other end. Motivational direction can also be assessed via psychophysiology by measuring the amplitude of muscle activation of the orbicularis orbi muscle in response to a sudden stimulus (Bradley & Lang, 2007). This measure is referred to as the eye blink startle response or eye blink startle reflex; the stimulus used to elicit it is referred to as a startle probe. In all of the studies described in this dissertation, the startle probe was an auditory probe which was a loud, brief burst of white noise. The startle reflex in response to an abrupt noise follows a simple pathway from the cochlear nucleus to the pontine reticular formation; the latter structure has efferents to spinal and cranial motorneurons which project to the reflex effectors. Inhibitory and excitatory projections from other brain nuclei to the pontine reticular formation are responsible for the modulation of this reflex (Koch & Schnitzler, 1997).

One of the important ways in which the magnitude of the startle response is modulated is by emotional valence. This affective modulation of startle is considered a well-validated and widely accepted psychophysiological measure capable of distinguishing between the approach-avoidance dichotomy of emotions (Amodio & Harmon-Jones, 2011; Vrana, Spence, & Lang, 1988). The startle reflex is modulated by the motivational system (Lang, Davis, & Öhman, 2000), and shows an increased amplitude when experiencing fear (Globisch, Hamm, Esteves, & Ohman, 1999; Hamm, Cuthbert, Globisch, & Vaitl, 1997) and when experiencing other unpleasant emotions (Vrana et al., 1988) as compared to startle measured during neutral emotional states. In contrast, when presented with pleasant stimuli, startle amplitudes are relatively reduced. Affective modulation of the startle reflex magnitude results from activation of a variety of structures in which the amygdala plays a pivotal role. This modulatory effect of the motivational neuro-circuitry on the eye blink motor reflex is described in more detail in the literature (e.g., M. Davis, 2006; Lang, Bradley, & Cuthbert, 1998; Misslin, 2003). This affective modulation of startle is only evident when the emotional states are sufficiently arousing or intense (Bradley, Codispoti, Cuthbert, & Lang, 2001). Apart from affective modulation startle can also be modulated by attention and arousal, which we will not go into now, but which we will address in the discussion section.

Although higher startle amplitudes following manipulations that induce fear or unpleasantness are a robust finding, this affective modulation has predominantly been tested using visual (e.g., Jansen & Frijda, 1994; Schupp, Cuthbert, Bradley, Birbaumer, & Lang, 1997), auditory (Bradley & Lang, 2000) and olfactory (Ehrlichman, Brown, Zhu, & Warrenburg, 1995) stimuli, but not during aversive interoceptive stimulation. Therefore, the exploration of the startle topography during background interoceptive stimulation takes on a predominant role in this dissertation.

CHAPTER 3

Aims

The major aim of this doctoral project is to test whether the human eye blink startle paradigm can be used to measure defensive response mobilization during background interoceptive stimulation. The eye blink startle is a validated and widely used measure of affective state and defensive responding when used in the context of affective visual, auditory, and olfactory stimuli. The popularity of this paradigm is due to a number of reasons. (a) Startle is one of the few peripheral psychophysiological measures that are modulated by stimulus valence. (b) It can be measured in both animals and humans. (c) It bypasses the drawbacks inherent to self-report. (d) It can theoretically be used to measure covert defensive motor preparation and aspects of fear, which are not reflected in self-report and sympathetic activation alone. (e) And of course, another factor of major importance is that the neurobiology behind the reflex has already been well elucidated. In light of all this, it is relevant to know whether the startle paradigm can also be applied to interoceptive stimuli. If so, this would mean we can rely on a wealth of existing findings and theories based on visual, auditory, and olfactory stimuli, anticipation thereof, and mental imagery.

A two-step approach was used to address the aim of testing whether startle can be used as a measure of defensive response mobilization during interoception. In a first step, the concept interoception is thoroughly and critically reviewed, in order to come to a working definition of interoception to be used throughout this dissertation. In a second step, a series of explorative studies are conducted using a variety of stimuli, which are interoceptive according to the working definition proposed in the first step. In this series of explorative studies, in addition to measurements of startle, participants completed subjective rating scales. Moreover, skin conductance was measured in half of the studies as an index of sympathetic and emotional arousal.

3.1 Review: On the origin of interoception

Interoception is a term that came into existence in the previous century. Since its conception, there has been a shift in its meaning, but this shift has not yet been universally adopted, and has led to a variety of conceptualizations which often only slightly differ from one another. Due to the lack of consensus, it was necessary to arrive at a working definition of interoception. In line with the general trend in the literature of recent years to use the concept interoception as an inclusive term, it was opted to adopt interoception as an umbrella concept to refer to the subjective perception of the state of the body. Through which sensory channel this subjective perception arises does not matter. Even if

the perception of the body state is inaccurate or illusory, it is still an existing perception in the phenomenological experience of the perceiver. Although interoception is used in an extremely inclusive sense, the review does recognize and value the different components that can contribute to interoception. Examples of levels at which sensations can be considered as being distinct from one another can be: whether sensations are exogenous or endogenous; whether the sensations stem from tissues with efferent autonomic nervous system (ANS) and enteric nervous system (ENS) or from somatic nervous system (SNS) innervation; and which afferent homeostatic pathways and basal processing structures are involved in the early processing. Each of these components are specifically labeled. This creates a rudimentary classification system that allows for comparing different interoceptive sensations, and determine on which of the listed aspects they are alike, and on which they differ.

3.2 Study 1: Effect of seated trunk posture

Previous research found startle potentiation during unpleasant gastric stimulation (Schächinger, Degen, & Beglinger, 2009), while research using dyspneic stimulation did not find this potentiation (Ceunen, Vlaeyen, & Van Diest, 2013; Pappens, De Peuter, Vansteenwegen, Van den Bergh, & Van Diest, 2012; Pappens et al., 2010). Gastric discomfort usually leads to flexion of the spine (Sikirov, 2003), while dyspnea leads to extension of the spine (Honig, 1990). It is also known that posture can affect emotion (Duclos et al., 1989; Flack, 2006; Riskind & Gotay, 1982) as well as startle magnitude (Price, Dieckman, & Harmon-Jones, 2012; Wielgosz, Repshas, Greishar, & Davidson, 2012).

Taking the above observations into consideration, a study was set-up to investigate whether posture could be responsible for the difference in startle observed during gastric stimulation as opposed to that during respiratory stimulation. Participants in this study had to assume three different postures for several minutes each; one posture with the spine flexed, one with the spine upright, and one with the spine extended. Eye-blink startle was measured during all three postures, and self-report was administered after each posture.

3.3 Study 2: Effects of cold pain and respiratory stimulation on startle in women

Affective modulation of startle makes the relative magnitude of the startle response a reliable measure of emotional state in response to affective visual, auditory and olfactory stimuli. While affective modulation of startle has also been observed in anticipation of interoceptive stimulation (e.g., Hubbard et al., 2011; Lang et al., 2011; Melzig, Michalowski, Holtz, & Hamm, 2008; Naliboff et al., 2009; Pappens et al., 2013; Twiss et al., 2009), startle measured during actual interoceptive stimulation had only been scarcely reported prior to this study. Startle during breathing with a resistance, during CO_2 inhalation, and during thermic pain stimulation have all been studied previously. The few studies on this suggest startle is paradoxically not elevated during interoceptive stimulation,

but rather reduced. We were interested to know how startle would behave at different time points during prolonged stimulation. An earlier study from our group suggested that startle progressively reduces in response to CO_2. There were also indications that the affective component of pain during immersion of the hand in cold water fluctuates depending on time since immersion, and thus it could be hypothesized that perhaps so does the startle. However, the effect of cold pain on startle over time had not been tested previously. For breathing against a resistance (loaded breathing), no data were available at the time on how startle behaves when loaded breathing occurs over a prolonged interval. Our study was the first to administer all three of these interoceptive stimuli within subjects.

3.4 Study 3: Effect of visceral pain on startle

Further attempting to elucidate the startle response topography to interoceptive stimulation, we opted for stimulation of the distal, visceral part of the esophagus at individually determined pain threshold. Previous studies on gastric stimulation did find a potentiation of startle. This gave rise to the question whether startle at other sites along the alimentary tract would behave alike, or whether they would show the reduced startle as found in response to aversive respiratory stimuli. The esophagus was chosen as site of stimulation as it has clearly distinct somatic and visceral innervation, and allows for visceral stimulation without stimulating overlying somatic tissue.

Of lesser importance, yet of some interest was whether the phenomenon of affective pain modulation can be extrapolated to visceral pain. Another minor aim was to find out whether affective state is still reflected in startle in the context of visceral pain, as visceral pain itself was expected to induce a negative emotional state.

The major and minor aims of this study were addressed in a study which included six time intervals –i.e., blocks – were affect was induced with a series of affective pictures of one valence per block. Half of these picture blocks were with, and half without the esophageal stimulation. A seventh picture free block with esophageal stimulation was added in order to observe the effect of visceral pain in the absence of concurrent visual mood stimuli. Startle and skin conductance were measured in each block, and self-report at the end of each block assessed subjective fear, valence, arousal and pain intensity.

3.5 Study 4: Visceral fear learning

While earlier research as well as our own research suggests that startle potentiation does not occur for a range of aversive interoceptive stimuli, other research suggests that during interoceptive conditioning (IC), startle potentiation does occur (Pappens, Smets, Vansteenwegen, van den Bergh, & Van Diest, 2012; Pappens et al., 2013). In these IC studies startle potentiation occurred in response to an interoceptive stimulus when this stimulus immediately precedes a more aversive interoceptive sensation.

Visceral fear learning through IC has been proposed as a mechanism involved in the etiology and maintenance of somatoform disorders (Acheson, Forsyth, & Moses, 2012; Acheson et al., 2007; Bouton et al., 2001; Craske et al., 2011; De Peuter et al., 2011; Mayer, 2000; Meulders et al., 2011; Pappens et al., 2013; Zaman, Vlaeyen, Van Oudenhove, Wiech, & Van Diest, 2015). Most relevant to somatoform disorders and medically unexplained symptoms is when CS and US are both in the same sensory mode; this is homoreflexive IC. However, the few studies on homoreflexive IC prior to this study have only used respiratory stimulation.

The purpose of the current study was to establish a homoreflexive interoceptive conditioning paradigm using esophageal stimulation at two different intensities; one at detection threshold (CS) and another at a subjectively painful intensity (US). In a between subject design, we also included a control group which was administered the same stimuli, but with a different temporal contingency. The primary information on participants' subjective expectancy of the US was obtained by having participants continuously rate the extent to which they expected the US to occur. Skin conductance was measured as an indication of arousal. In line with the doctoral project's main aim, startle was included as a measure so we could further map the startle response topography to interoceptive stimuli; in this case in response to conditioned interoceptive stimuli, prior to learning of associations, during learning, and during extinction. Because study 3 found gender differences in startle in response to esophageal stimulation, this study was optimized to investigate potential gender differences, and had an equal ratio of men to women.

Part II

Startle in Interoception

CHAPTER 4

Effect of Seated Trunk Posture on Eye Blink Startle and Subjective Experience: Comparing Flexion, Neutral Upright Posture, and Extension of Spine

Ceunen, Zaman, Vlaeyen, Dankaerts, Van Diest (2014). *PloS one, 9*(2), e88482. Doi: 10.1371/journal.pone.0088482

Abstract:

Postures are known to be able to affect emotion and motivation. Much less is known about whether (affective) modulation of eye blink startle occurs following specific postures. The objective of the current study was to explore this. Participants in the present study were requested to assume three different sitting postures: with the spine flexed (slouched), neutral upright, and extended. Each posture was assumed for four minutes, and was followed by the administration of brief self-report questionnaires before proceeding to the next posture. The same series of postures and measures were repeated prior to ending the experiment. Results indicate that, relative to the other postures, the extended sitting posture was associated with an increased startle, was more unpleasant, arousing, had smaller levels of dominance, induced more discomfort, and was perceived as more difficult. The upright and flexed sitting postures differed in the level of self-reported positive affect, but not in eye blink startle amplitudes.

1. Introduction

Both dynamic and static body posture is understood to serve a communicative role, as does verbal content, vocal tonality, vocal volume, and facial expression (de Gelder, 2006). Darwin (1872) already documented that body posture communicates emotional states. This is understood to be a consequence of different emotions having different effects on body posture (Oosterwijk, Rotteveel, Fischer, & Hess, 2009). Interestingly, the association between emotions and body posture is not merely unidirectional. Several studies indicate that posture also has feedback and regulatory effects on emotion and motivation (Duclos et al., 1989; Flack, 2006; Riskind & Gotay, 1982).

The reciprocal influence of posture and emotion may have relevant implications for well-being and for research on emotion. It is plausible that the emotional well-being of office workers worldwide is affected by frequently sitting in a slouched posture for extended periods of time given the documented effects of such a posture (Peper & Lin, 2012), potentially resulting in more than just back pain. Apart from well-being, research on emotion may need to control for the posture of participants, even if posture is not the main variable of interest. Indeed, posture is potentially a confounding variable; it can affect outcomes of similar studies on emotion differently (Price, Peterson, & Harmon-Jones, 2012). As such, the body posture-emotion association is an avenue of research that needs further investigation.

Research on how emotion is affected by posture has primarily been investigated via self-report (e.g., Duclos et al., 1989; Flack, 2006) and behavioral task performance (e.g., Riskind & Gotay, 1982), but need not be limited to these measures. One well-established physiological measure of emotion is affective modulation of eye-blink startle (Bradley & Lang, 2007). The eye blink startle reflex consists of the activation of the orbicularis oculi muscle surrounding the eye in response to a startling stimulus. This is usually a short burst of white noise and is referred to as an auditory startle probe. The magnitude of the startle response is modulated by emotional valence, and is considered a well-established psychophysiological measure capable of distinguishing between the approach-avoidance dichotomy of emotions (Amodio & Harmon-Jones, 2011; Vrana et al., 1988). With presentation of pleasant stimuli triggering approach motivation, the startle magnitude in response to an auditory startle probe is reduced relative to a neutral emotional state, whereas it is increased when aversive stimuli related to avoidance motivation are presented. Although this emotional modulation is a robust finding in response to a varied range of emotional stimuli (Bradley & Lang, 2007), the effect of posture on emotional modulation of startle has received scant attention thus far.

At the time of writing, we know of only one published research paper addressing modulation of startle in relation to posture (Price, Dieckman, et al., 2012). It reports on a study that examined the effect of posture on startle during exposure to pictures high in approach motivation versus neutral

pictures, matched for content. Results indicated that leaning forward – the posture most congruent with the approach motivation pictures (Price & Harmon-Jones, 2011) – increased the relative inhibition of startle magnitude in response to approach related pictures more so than did a reclining posture.

One other, unpublished study by Wielgosz and colleagues (2012) demonstrated an interaction between posture and presence of threat. Assuming a 'protective' posture (i.e., shoulders shrugged) in a context with threat of mild electric shocks helped to decrease startle magnitude relative to assuming an open posture (i.e., shoulders drawn back) in the same threatening context. In the threat-free context, however, the protective posture elicited an increase in startle magnitude relative to startle elicited during an open posture in that same threat-free context. Additionally, the outcomes indicated that increased effort associated with holding either a protective or open posture for several minutes led to increased startle magnitude relative to magnitudes measured during the minutes in which a more effortless neutral upright posture was held, regardless of context.

It is interesting to note that Price, Dieckman and Harmon-Jones (2012) classified postures based on approach motivation and compared the difference between inclining and reclining, whereas Wielgosz and colleagues (2012) classified postures based on anticipation of threat by comparing the use of the shoulders in protecting versus exposing oneself. Inclination of the upper body versus reclination, and shoulder positioning are obviously not the only variables in posture that relate to emotion. The sagittal position of the spine (in coordination with position of head and shoulders) is another postural variable frequently associated with emotion. Specifically, flexion of the spine and protraction of head and shoulders (i.e., slumping/slouching) is associated with unpleasant, sad emotional states, whereas extension of the spine and retraction of head and shoulders (arching the back, sticking out the chest) is associated with positive affect and/or an (over)confident state of mind (Briñol, Petty, & Wagner, 2009; Coulson, 2004). In particular, the contrasting emotions associated with flexion versus extension of the spine, makes variation of spinal posture an interesting variable for research on the effect of posture on emotion.

Although no research has previously been conducted on startle modulation in response to manipulation of spinal posture in the sagittal plane, the idea for the current study did not arise in a vacuum. Contrasting flexion and extension of the spine and observing their effect on startle was inspired by previous findings on startle in relation to unpleasant bodily sensations. Whereas unpleasant gastric stimulation appears to be associated with startle potentiation (Schächinger et al., 2009), increasing evidence suggests that dyspneic stimulation is not (Ceunen et al., 2013; Pappens, De Peuter, et al., 2012; Pappens et al., 2010). We hypothesized that the contrasting findings with the unpleasant bodily stimulations could be due to spinal posture associated with these two different

types of stimulation, or with associated tension in muscles that regulate spinal posture. Whereas dyspnea is associated with spine extension (Honig, 1990), stomach ache instinctively leads to spine flexion, a posture associated with easing of gastro-intestinal function (Sikirov, 2003). The hypothesis that posture could be responsible for the difference in results in these two types of bodily stimuli was reinforced by the notion that body posture affects emotion (as discussed above), which in turn is known to modulate startle. Additionally, the notion that startle is associated with flexion of the spine in the whole body startle (Landis & Hunt, 1939; Yeomans, Li, Scott, & Frankland, 2002) may imply that posture prior to and during startle has the potential to modulate not only the whole body startle as observed earlier (Brown, Day, Rothwell, Thompson, & Marsden, 1991), but also the eye blink startle magnitude.

To investigate this hypothesis, we set up the current study. The main aim was to explore whether different spinal sitting postures affect self-reported emotion and eye blink startle differently, with the postures under investigation being a flexed, a neutral upright, and an extended spine. As discussed earlier, the effort associated with postures can affect startle magnitude regardless of the specific postural manipulation (Wielgosz et al., 2012). For this reason we also included questions on discomfort experienced, and difficulty maintaining each specific posture, both reflecting emotional correlates of effort.

2. Materials and Methods

2.1. Participants

Thirty-six psychology freshmen (mean age = 19.44 years, range 18-30 years, 29 women) participated in return for course credit. Exclusion criteria were pain-related conditions (lower back pain, stomach ache, or others), known or obvious abnormal kyphosis, lordosis, or scoliosis, presence or history of psychiatric disorders and/or epilepsy, and current usage of psychopharmacological agents.

2.2. Ethics Statement

Prior to participation, all subjects read and signed an informed consent: the consent guaranteed anonymity, and stated that participation was voluntary and could be terminated at any point in time without loss of the promised course credit. The study had been approved by both the Psychological and Medical Ethical Committees of the University of Leuven, Belgium and was in accordance with the Declaration of Helsinki (World Medical Association, 2008).

2.3. Sitting postures

Each sitting posture was defined by four key points that had to be respected. These were: (1) the position of the pelvis, (2) the position of the upper back, (3) the position of the head, and (4) the position of the shoulders. (See *Figure 3*)

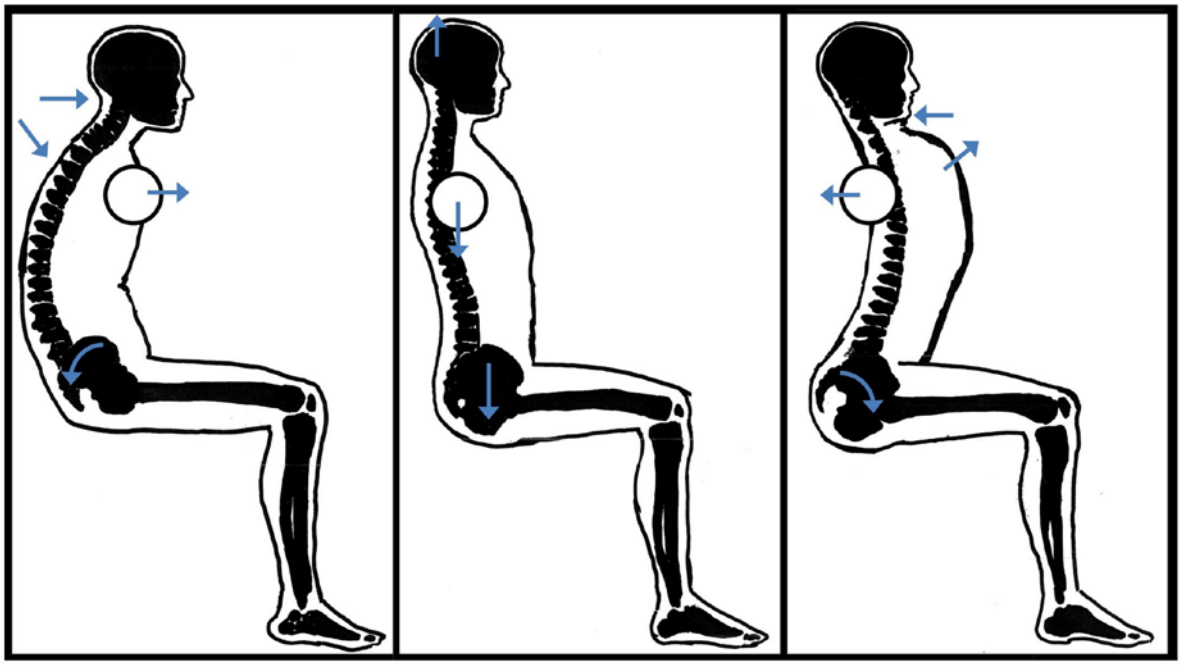

Figure 3. Postural manipulations. These illustrations accompanied the verbal instructions for the flexed (slouched), neutral upright, and extended posture, displayed here respectively from left to right. Negatives of these three illustrations (black background, white figures) were shown one at a time. Arrows appeared one by one during verbal instruction to highlight the four key points that had to be respected.

2.3.1. Flexed posture

In order to have the spine in a flexed position, participants were asked to perform a posterior pelvic tilt, and to curve the upper back into maximal kyphosis. Additionally, both the head and the shoulders had to be protracted. Participants were instructed to assume this posture without exerting the abdominal muscles needlessly, as each posture had to be held for four minutes on end.

2.3.2. Upright posture

In order to have participants assume a neutral upright sitting posture with normal curvature of the spine, participants were asked to position their pelvis neutrally by sitting straight on their sitting bones, and by 'pulling' their head upward from the crown. Shoulders were held next to the body in a relaxed (as opposed to shrugged) position. Attention was paid that the upper back was neither slouched forward (kyphotic), nor curved backward (hyperextended).

2.3.3. Extended posture

To sit in an extended spinal posture, subjects performed an anterior pelvic tilt, curved their upper back in a posterior direction, and retracted head and shoulders.

2.3.4. Manipulation check

The experimenter, although in another room, was able to monitor the participants' overt compliance with the instructions by means of a closed-circuit video monitoring system. Additionally, three blinded observers retrospectively conducted a forced choice task of classifying still shots of all six postures (3 x 2) of each participant. These still shots were extracted from a video-recording device which was positioned laterally on the left hand side of the participant. To ensure anonymity of participants, a black oval was inserted on the profile view of their face while leaving the backside of the head visible in order to allow the blinded observers sufficient detail to score the postures correctly.

2.4. Self-report measures

At the end of each posture a computerized 9-point scale of the language-free Self-Assessment Manikin (SAM-Scale, Bradley & Lang, 1994) and computerized Borg scales were administered. On the SAM the subjects had to retrospectively rate the mean valence (unpleasant = 1; pleasant = 9), arousal (calm = 1; excited = 9), and dominance (lack of control = 1, sense of control = 9) they had experienced while adopting the specific posture they had most recently assumed. Borg Scales for perceived mean discomfort and mean difficulty during the posture ranged from 0 to 10 and were labeled from none (0) to maximal (10). After each posture and after filling in the computerized SAM and Borg scales, subjects had to answer a paper and pen version of the Dutch version of the Positive And Negative Affect Schedule (PANAS)-state questionnaire (Engelen, De Peuter, Victoir, Van Diest, & Van den Bergh, 2006).

2.5. Somatic reflex measurement and processing

2.5.1. Eye blink startle response

Eye blink startle responses were elicited by binaural acoustic presentations of short bursts (50ms) of white noise (95dB). Two electrodes filled with high conductivity Microlyte electrolyte gel measured the electromyographic (EMG) activity of the left orbicularis oculi muscle as a response to the acoustic startle probes at the sites specified by Blumenthal et al. (2005); a ground electrode was placed on the center of the forehead. To reduce inter-electrode resistance, all sites were first cleaned with alcohol. The EMG signal was amplified by a Coulbourn isolated bioamplifier (LabLinc v75-04) with a 13HZ high pass, and 1 KHz low pass bandpass filter. This signal was then routed to a Coulbourn integrator (LabLinc v76-24), which rectified and smoothed the signal with a time constant of 20ms. The startle EMG was sampled at 1000Hz and recorded starting from 500ms prior to probe onset, until 1000ms after probe onset.

2.5.2. Software

A 16-Bit National Instruments PCI-6221 data acquisition card (National Instruments, Austin, Texas) transmitted the EMG signals from the Coulbourn modules to a computer. Affect 4.0 software (Spruyt,

Clarysse, Vansteenwegen, Baeyens, & Hermans, 2010) was used for timing the presentation of startle probes as well as for data acquisition. A program named PSychoPHysiological Analysis, abbreviated as PSPHA (De Clercq, Verschuere, De Vlieger, & Crombez, 2006) was used to handle the recorded signals offline and to extract the relevant parameters necessary for statistical analysis.

2.6. Procedure

We created six groups, as six orders of presentation were possible based on the three postures (the orders were FUE, FEU, EUF, EFU, UEF, and UFE, with F = flexed, U = upright, and E = extended). Participants were randomly assigned to one of these groups, with the constraint that there were equal numbers of participants assigned to each group. An attempt was made to keep the ratio of men for all six posture orders approximately equal, with one male participant per group in five out of six groups, and two males in a sixth group. Upon arrival, the experimenter provided participants an informed consent, which they were requested to read and sign. Next, EMG electrodes were attached – subjects were informed that these were meant for measuring physiological responses, albeit without further specifications. The experimenter then verbally went through the experimental procedure, assisted by on-screen, step-by-step depictions of each of the postures and their key points, and also each of the computerized self-report scales. Additionally to onscreen depictions, key points for each posture were demonstrated by the experimenter (pelvic ~, upper back ~, head ~, and shoulder positions), followed by a request to the participant to briefly assume the posture. The latter was done in order for the experimenter to assess whether participants were able to correctly assume the desired postures. Participants were told that the computer monitor would display when to assume which particular posture. The time for each posture started ticking only after subjects had assumed the posture. Participants were also told to keep their gaze in the direction of the computer monitor on which a fixation cross would appear throughout the experiment. If participants indicated they had no further questions, headphones were placed on their ears, and the experimenter left to the adjacent operator room. Lights remained on (not dimmed) throughout the entire experiment.

The experiment started with a habituation phase in which 10 startle probes were administered to reduce the effect of novelty of startle probe on startle magnitude (Blumenthal et al., 2005). After this habituation phase, startle probes were presented on average every thirty seconds during a posture, although the exact time of administration was kept variable. While keeping their gaze at a fixation cross on a computer screen, each of the three postures was assumed for four minutes, with eight startle probes delivered per posture. Once a minute, shortly after administration of a startle probe, a picture of the posture the participant was expected to continue assuming, appeared on the computer screen to remind the participant of each of the key points (pelvic ~, upper back ~, head ~, and shoulder positions) indicated by arrows embedded in the picture. After each posture and before

continuing to assume the next posture, participants rated the aforementioned self-report scales. Once all three postures were assumed a first time, the same three postures were repeated a second time in exactly the same order of presentation, while again rating all self-report questions after each posture.

2.7. Data analysis

2.7.1. Manipulation check

The labels given by each of the three raters were checked on the percentage of postures misidentified. We also checked if the postures of specific participants were misidentified by more than one rater. An inter rater reliability analysis using the Fleiss Kappa statistic was performed to determine consistency among raters in identifying the three postures.

2.7.2. Eye blink startle response

Eye blink startle EMG responses were calculated by subtracting the mean baseline value (0 to 20 ms after probe onset) from the peak value found in the 21 to 175ms time window after probe onset. Startles measured during habituation were excluded from data analysis. EMG measures were visually inspected for presence of spontaneous blinks or other phasic muscular tension of the orbicularis oculi muscle present at the onset of the startle probe and rejected if necessary. As a result, three participants (1 male, 2 females) were excluded since 30% or more of their startles were rejected. Because we were interested in intra-individual differences in response amplitude and not in inter-individual differences, startle probes were transformed to T-scores (Blumenthal et al., 2005). Mean startle amplitudes were calculated for each posture per participant per series. Analysis was performed using SPSS 20. Linear mixed models analysis was performed. In order to test the effects of posture, two dummy variables were created, one coding for the flexed (D_Flexed 0/1) and one for extended posture (D_Extended 0/1). The upright posture served as reference (reference coding) (Aiken & West, 1996). The model had mean startle T-scores as criterion variable and as continuous predictors Valence, Arousal, Dominance, Discomfort, Difficulty, Negative affect and Positive affect; as categorical predictors Block (1/2), Flexed posture (0/1) and Extended posture (0/1) were included. All continuous predictors were centered on the person's mean (Aiken & West, 1996; Van Breukelen & Van Dijk, 2007). A repeated measures random effects defined by a Block*Position interaction was included. Compound Symmetry was preferred over Unstructured as covariance structure (X^2_{19} = 27.018, p = .104) as an increase in model complexity did not result in a significant better fit). For the regression parameter estimates, standardized Beta's are reported, unstandardized Beta's and Cohen's *d* are displayed in Table 1. Cohen's *d* values larger than .2, .5 and .8 are respectively described as small, medium and large effect sizes (Cohen, 1988).

2.7.3. Self-report

Analysis of the effect of posture on the self-report measures was done using STATISTICA 10. An α-level of .05 was set for statistical significance and partial squared éta effect sizes (η_p^2) are reported. Greenhouse-Geisser corrections for violation of sphericity were applied when appropriate. Excluded participants from the startle analysis (see Section 2.6.1.) were also omitted from the self-report analysis. The measures of perceived valence, arousal, dominance, discomfort and difficulty were all separately entered into a 3x2 repeated measures ANOVA with POSTURE and SERIES as within subject variables. Of the PANAS-state, the Positive Affectivity (PA) score was analyzed separately from the Negative Affectivity (NA) score. Significant effects on any of the self-report items were further subjected to Tukey-Kramer post-hoc testing.

3. Results

3.1. Manipulation check

One observer had misidentified only 2% of all postures, while the other two observers each had misidentified only 1% of all postures. There was no overlap between observers on misidentifications: that is, any postures that were misidentified were only misidentified by one observer. Flexed postures were never misidentified. The inter rater reliability was found to be Kappa = 0.96 ($p<0.001$), 95% CI (92.19, 98.24).

3.2. Eye Blink Startle

The model significantly predicted startle amplitudes (X^2_{10} = 60.719, p <.001) compared to the most parsimonious model (no predictors, only intercept). There were no effects of Valence, Arousal, Discomfort, Dominance and Positive affect. There was a significant effect of Difficulty (β = -.742, t(165) = 2.3815, p = .018), suggesting that the more a posture was perceived as difficult the lower startle amplitudes were. There was a strong effect of Negative affect (β = 6.217, t(165) = 4.110, p < .001); the higher Negative affect scores were, the higher startle amplitudes were. Startle amplitudes habituated over time, as indicated by the negative beta of Block (β = -2.135, t(165) = 3.380, p = .001). As expected, there was an effect of posture on startle amplitudes. During an extended posture, startle amplitudes were significantly higher compared to an upright posture (β = 3.236., t(165) = 3.402, p = .001) and a flexed posture[5] (β = 2.502, t(164) = 2.845, p = .005), while the flexed posture did not differ from the upright posture (β = .746, t(165) = .1.032, p = .304). See table 2 for details.

[5] This was analyzed through an identical model apart from the dummy variable (D_Flexed), which was replaced by a dummy variable for an upright posture (D_Upright), so that the flexed posture served as reference.

Table 2.

The estimates (β), standard errors (SE), flagged significances and Cohen's d for the multiple regression with reference (dummy) coding.

	β	***SE***	***d***
Intercept	51.571***	1.026	*7.823*
Valence	-.003	.376	-
Discomfort	-.322	.336	-
Difficulty	-.742*	.312	*.37*
Arousal	.150	.268	-
Positive affect	.-.181	.896	-
Dominance	-.261	.263	-
Negative affect	6.217***	.1.513	*.64*
Block	-2.135***	..632	*.526*
D_Flexed	746	.722	-
D_Extended	3.236***	. 951	*.53*

Note. Reported β's are unstandardized. Effect sizes of parameter estimates are reported as Cohen's *d*. ***: p ≤ .001, **: p ≤ .01, *: p ≤ .05

3.3. Self-report

Repeated measures ANOVAs with POSTURE and SERIES as within subject variables, indicated a main effect of POSTURE for valence, $F(2, 64) = 32.17$, $p < .001$, $\eta_p^2 = .50$, arousal, $F(2, 64) = 9.91$, $p < .001$, $\eta_p^2 = .24$, dominance, $F(2, 64) = 9.57$, $p < .001$, $\eta_p^2 = .23$, discomfort, $F(2, 64) = 35.48$, $p < .001$, $\eta_p^2 = .53$, difficulty, $F(2, 64) = 36.48$, $p < .001$, $\eta_p^2 = .53$, and both the PA, $F(2, 64) = 4.32$, $p = .02$, $\eta_p^2 = .12$ and NA items of the state PANAS, $F(2, 64) = 4.44$, $p = .02$, $\eta_p^2 = .12$. Further Tukey-Kramer post-hoc testing indicated that the extended posture was significantly more unpleasant, more arousing, had smaller levels of dominance, induced more discomfort, and was perceived as more difficult (for all items, $p < .002$) than both of the other postures which were never significantly different from one another. Regarding the PANAS, post-hoc tests indicated that an upright posture was associated with significantly more PA than a flexed posture ($p = 0.02$). (The level of PA associated with the extended posture did not significantly differ from either that of the upright or flexed posture). NA was significantly higher in the extended posture than in the flexed posture ($p = 0.01$), but NA during the upright posture was not significantly different from either extended or flexed posture. See table 3 for details.

There was also a main effect of SERIES for difficulty, $F(1, 32) = 8.01$, $p = .008$, $\eta_p^2 = .20$, with the second series of postures being perceived as more difficult than the first. A main effect of SERIES was also present for PA, $F(1, 32) = 14.72$, $p < .001$, $\eta_p^2 = .32$ of the PANAS, with less PA during the second series. No other main or interaction effects were found.

Table 3.

Means and standard deviations for overall valence, arousal, dominance, discomfort, difficulty, Positive Affect (PA) and Negative Affect (NA) experienced during each of the three postures.

	Flexed	Upright	Extended
SAM – Valence (1 = unpleasant, 9 = pleasant)	4.45^{a} (1.77)	5.12^{a} (1.65)	2.56^{b} (1.04)
SAM – Arousal (1 = calm, 9 = aroused)	3.36^{a} (1.86)	3.67^{a} (1.66)	4.74^{b} (1.89)
SAM – Dominance (1 = not dominant, 9 = dominant)	5.17^{a} (1.79)	5.52^{a} (1.60)	4.24^{b} (1.79)
Borg – Discomfort (0 = none, 10 = maximal)	3.33^{a} (1.77)	2.82^{a} (1.49)	6.02^{b} (2.16)
Borg – Difficulty (0 = none, 10 = maximal)	2.97^{a} (1.66)	2.55^{a} (1.70)	5.38^{b} (2.05)
PANAS – PA (1 = very little, 5 = a lot)	2.17^{a} (0.73)	2.3^{b} (0.71)	2.21^{ab} (0.72)
PANAS – NA (1 = very little, 5 = a lot)	1.26^{a} (0.34)	1.31^{ab} (0.43)	$1,37^{b}$ (0.39)

Note. SAM values of 5 are considered everyday baseline levels of respectively valence, arousal and dominance. Means in the same row which share a subscript are not significantly different from one another according to Tukey-Kramer post-hoc tests. Standard deviations are indicated by the numbers between brackets.

4. Discussion

The current study was an exploration of the effects of spinal posture on subjective experience and eye-blink startle. So far, only one prior publication (Price, Dieckman, et al., 2012) and one unpublished study (Wielgosz et al., 2012) included startle as a primary dependent variable in research on the bottom-up effects of posture on emotion. These studies respectively manipulated inclination versus reclination of the upper body, and shoulder positioning. To our best knowledge, our study was the first to systematically manipulate the spinal posture on a sagittal plane during sitting. It included flexion and extension of the spine, as well as an additional upright posture with neutral spinal curvature. All three postures were held for four minutes each, and then repeated a second time. A blinded manipulation check suggests all participants assumed the postures correctly. That the accuracy of identification was slightly less than 100% is presumably due to a combination of clothing

and camera angle masking the extended curvature of the back, thereby reducing visual differences between upright and extended postures on the images.

Using these postural manipulations, we found that the extended posture was associated with significantly increased subjective unpleasantness, arousal, discomfort, and difficulty, and a decreased level of dominance relative to both other postures. The extended posture was also characterized by increased state NA relative to the flexed posture. Other than the extended posture, the upright posture was associated with higher state PA relative to the flexed posture. Repeating the series of all three postures a second time led to a decrease in PA, and an increase in perceived difficulty.

As for startle, the extended posture was associated with an increase in startle amplitude relative to both other postures. Increased state NA also led to increased startle, regardless of posture. Increased difficulty paradoxically led to a decrease in startle when all other factors were held constant, even though the extended posture had both the highest mean startle and highest mean difficulty. That eye blink startle amplitude is smaller when difficulty increases, appears to be contrary to the well-documented increase in startle magnitude that is typical for unpleasant emotional states (Bradley & Lang, 2007). On the one hand, this could be an indication that difficulty is not necessarily related to negative affect. On the other hand, we need to take into account that reduction in startle amplitude is not necessarily indicative of an absence of unpleasant affect. Several studies suggest that orientation of attention to bodily sensations reduces responsivity to an auditory startle eliciting probe (e.g., Deuter et al., 2012; Filion, Dawson, & Schell, 1998; Pappens, Van den Bergh, & Van Diest, 2011b). Sensations of muscle tension and effort associated with postures perceived as difficult, may shift attention to bodily sensations and can as such be responsible for the observed reduction in startle responsivity with difficult postures – even if such postures induce unpleasant affect.

From the self-report data, the affective changes occurring in conjunction with the extended posture are suspected to be predominantly due to the unpleasant effort associated with it, and not due to pre-existing associations with that body posture. If the affective changes were due to the meaning associated with the body posture, then the flexed posture should be standing out as most negative (Peper & Lin, 2012), not the extended posture. The assumed extended posture is a rather unnatural sitting posture, especially considering that 'sticking the chest out' was not performed in isolation, but in combination with an anterior pelvic tilt. Because the resulting posture is unnatural, any negative affect resulting from such a posture is unlikely to be due to associations with that particular body posture, and is most probably due to the effort needed to assume and maintain that posture.

Our data do provide some evidence that pre-existing emotional body posture associations may also exert an effect on affective state when assuming a body posture. In support of this, we like

to point out that PA scores were significantly lower during the flexed, i.e. slouched posture as compared to sitting upright. This finding can be interpreted as an indicator of a pre-existing association of PA with sitting upright in our participants, and the absence thereof when sitting in a slouched position.

These conclusions as inferred from our data suggest avenues for future research. Our study suggests that any uncomfortable, unnatural posture will evoke higher startles and more unpleasantness than postures with a pre-existing association with an aversive, negative emotion. This is an assumption that can be tested relatively easily after identifying other uncomfortable, unnatural postures. These postures can then be contrasted to postures used in expressing negative emotions. Given that unnatural postures require activation of muscles that are relatively untrained, such postures may be suspected to induce an unpleasant affective state by eliciting muscle soreness and perhaps some level of discomfort or pain. For this reason, we advise that future studies evaluating startle reflex include post hoc questions on whether pain was experienced during the posture, and if so, to which extent.

In future studies, it would be of additional interest to find a physiological correlate that is able to measure the effect of postures on emotion, which are due to pre-existing body posture associations, rather than due to effort. Our study suggests that PA remains relatively unaffected by unpleasant effort, and is likely the result of pre-existing emotional associations with specific postures. One method for detecting PA physiologically regardless of arousal is by measuring the post-auricular reflex (Gable & Harmon-Jones, 2009). Including this measure in future research may be more fruitful in paradigms that are primarily concerned with the effect of different body postures on emotion that are not due to effort, but due to pre-existing body posture associations.

Further implications of our findings are that future studies aimed at pinpointing the effect of embodiment on emotion, particularly on negative emotions, should try to devise postural manipulations that keep the required effort associated with the different postures equal and as minimal as possible. A more general implications is that future studies on emotion with no particular focus on postural manipulations, should at all costs avoid to have their subjects positioned in an effortful posture in order to limit confounding.

In conclusion, our findings underscore that posture, and especially the effort associated with adopting a specific posture affects both the affective state and eye blink startle magnitude of individuals. We hope that emotion researchers take note that any strenuous posture may affect their results thus should be avoided, unless a strenuous posture is the manipulation under investigation. If emotion is the subject of the study and a strenuous posture cannot be avoided, then care needs to be taken in interpreting the results. As up to now inclusion of psychophysiological measures such as

startle in research on the effects of posture is relatively scarce, we consider our conclusions to be preliminary and in need of further testing, replication and extension using the same and other psychophysiological measures of emotion, as well as a variety of postural manipulations.

CHAPTER 5

Atypical modulation of startle in women in face of aversive bodily sensations

Ceunen, Vlaeyen, Van Diest (2013). *International journal of psychophysiology, 88*(2), 157-163. Doi: 10.1016/j.ijpsycho.2013.03.013

Abstract:

Eye blink startle magnitude is assumed to be higher in threatening contexts. A scarce amount of studies suggest this does not hold true when startle is measured during perceived threats to homeostatic integrity. The present study was set up to describe the startle response pattern to a selection of interoceptive stimuli. Female subjects (N=36) were exposed once to 90 seconds of continued (1) cold pain, (2) inhalation of a gas mixture of 7.5% CO_2, and (3) breathing against an inspiratory and expiratory resistive load. Each stimulus was preceded and followed by a 90 seconds period of rest, respectively labeled baseline and recovery. Even after correcting eye blink startle responses for habituation, a decreased startle amplitude was evident during these stimuli. Results suggest that startle amplitude during aversive stimulation is inversely correlated with perceived fearfulness for women, although further studies are necessary to corroborate this interpretation.

1. Introduction

Interoception, the perception of the state of the body, serves to maintain homeostasis and is closely linked to the experience of emotions (Craig, 2002). *Interoceptive fear* is the apprehension of bodily sensations (Shear et al., 1997) and can manifest itself following the perceived disruption of homeostasis or in the anticipation thereof (Furst & Cooper, 1970). The anticipated or perceived disruption of homeostasis that lies at the heart of interoceptive fear, can potentially relate to any part of the organisms' functioning, including gas-exchange and thermoregulation. Interoceptive fear includes fear of pain, as pain is a perception related to the body state, processed in a neural network that largely overlaps with processing of non-painful interoceptive sensations (Legrain, Iannetti, Plaghki, & Mouraux, 2011; Moseley, Gallace, & Spence, 2012), and in that painful stimulation is relayed through a central homeostatic pathway along with other visceral and somatic afferents signaling disruption of homeostasis (Craig, 2003).

From an evolutionary perspective, fears promote an animal's chances of survival by helping to select a response appropriate for counteracting a perceived or anticipated threat (Ohman & Mineka, 2001). In this line of logic, interoceptive fear can have an adaptive advantage in urging a behavioral response to restore homeostasis or prevent its disruption. However, interoceptive fear in the absence of a real threat may paradoxically lead to over-perception of bodily sensations and to excessive physical symptom reports.

Functional disorders, anxiety disorders, and pain related disorders, affect a significantly large part of the population. In all of these disorders interoceptive fears play a key role, implying that the advancement of both clinical and fundamental knowledge on interoceptive fear is of utmost importance. A body of literature as well as a number of laboratory studies imply that the etiology and maintenance of such disorders is due to associative learning processes (Acheson et al., 2007; Bouton et al., 2001; De Peuter et al., 2011; Mayer, 2000; Meulders et al., 2011; Pappens et al., 2013). Because of interoceptive fear conditioning, originally benign sensations can elicit fear responses, when in the past these benign sensations have preceded an aversive interoceptive sensation.

Although interoceptive fear conditioning has a strong pedigree in the understanding of the aforementioned disorders, relatively little research has elaborated on the basic fear response topography to interoceptive stimulations used in the laboratory. Therefore, the major aim of the current study was to document unconditioned fear responding to such interoceptive stimulations. We made a selection of stimuli frequently used in experimental paradigms on pain (e.g., Helsen, Goubert, Peters, & Vlaeyen, 2011) and dyspnea (Acheson et al., 2007; Pappens, Van den Bergh, & Van Diest, 2011a), namely cold pain, inhalation of CO_2-enriched air, and loaded breathing. We selected these particular stimuli because a limited body of literature on startle in response to these stimuli is already

available, although as yet no design has presented these three stimuli in comparable manners within subjects. In this initial study, we limited ourselves to women: we justify this choice given that psychosomatic complaints and disorders have a higher prevalence amongst women (Kroenke & Spitzer, 1998; Şar, 2010).

The potentiation –i.e. the relative increase in magnitude– of the eye blink component of startle is a well-validated and widely accepted measure of fear responding. An important question relating to the aim of the current study is whether the eye blink component of startle can provide a reliable indication of fear *during* aversive interoceptive stimulation. The startle reflex is modulated by the motivational system (Lang et al., 2000), and shows an increased amplitude when experiencing fear (Globisch et al., 1999; Hamm et al., 1997) or something which is otherwise unpleasant (Vrana et al., 1988). Affective modulation of the startle reflex magnitude results from activation of a variety of structures in which the amygdala plays a pivotal role. This modulatory effect of the motivational neuro-circuitry on the eye blink motor reflex is described in more detail in the literature (e.g., M. Davis, 2006; Lang et al., 1998; Misslin, 2003). Although potentiation of startle following manipulations that induce fear or unpleasantness is a robust finding, it has predominantly been tested using visual and auditory stimuli. In contrast, the few studies on startle in response to aversive interoceptive stimulation present a more complicated and as yet inconclusive picture of findings.

With regards to thermal pain stimulation, findings are somewhat equivocal. For phasic heat pain, it appears that stimulation of short duration evokes startle potentiation (Crombez, Baeyens, Vansteenwegen, & Eelen, 1997), whereas stimulation of a longer duration does not (Horn, Blischke, Kunz, & Lautenbacher, 2012; Horn, Schaller, & Lautenbacher, 2012). For cold pain, there is an overall reduction when averaging startle amplitudes delivered at different times during a prolonged stimulation (Tavernor, Abduljawad, Langley, Bradshaw, & Szabadi, 2000), whereas such reduction may not be evident at individual time points (De Peuter, Ceunen, Van Diest, Van den Bergh, & Vlaeyen, 2009). Lovallo (1975) describes that pain in response to the Cold Pressor Test (CPT) does not keep rising progressively as time of immersion increases, a finding which may explain why startle probes at particular time intervals are not reduced.

Regarding dyspnea, findings from several studies conducted in our research group strongly suggest that dyspnea induced by the inhalation of CO_2-enriched air is associated with an inhibition of the startle reflex (De Peuter et al., 2009; Pappens, De Peuter, et al., 2012; Van Diest, Pappens, et al., 2009). Paradoxically, when dyspnea is induced by loaded breathing – a mechanical stimulus creating respiratory resistance – startle potentiation is evident when the stimulus is light (near perceptual threshold level), but absent when a respiratory load of higher (moderate) intensity is administered

(Pappens et al., 2010). This is paradoxical, because self-report measures as well as skin conductance indicated that the higher load was more aversive and arousing than the light load.

Possible mechanisms for these findings have been suggested by their respective authors, and will be reviewed in the discussion section. Regardless of the mechanism responsible for the apparently atypical startle pattern found in earlier studies documenting startle responding to the CPT, inhalation of CO_2-enriched air, and loaded breathing, it seems startle within one type of stimulus is inversely correlated with unpleasantness (Pappens et al., 2010). The following parsimonious conclusions could be made: (a) these types of aversive interoceptive stimulation are associated with a reduction in startle rather than potentiation. (b) As dyspneic stimuli become more aversive as time progresses, it could be expected that startle responsivity decreases overall as the duration of dyspneic interoceptive stimulation increases. However, (c) startle in response to painful peripheral hypothermic stimulation may be an exception in that pain fluctuates over the course of time, and accordingly, startle may not necessarily decrease linearly over time.

To test these hypotheses, in the current study we subjected these earlier findings to a novel experimental paradigm, allowing for a within-subject comparison of unconditioned defensive responding to these three types of sustained, aversive interoceptive stimulation. The primary aim of this study was to shed light on the startle response over time to three types of stimulation. Eye blink startle responses were studied during 90 s periods of cold pain, inhalation of CO_2-enriched air, and loaded breathing. In contrast to the studies of Pappens et al. (2010; 2011a), which applied loads for only one inspiration, the continued stimulation allowed for testing our assumption that startle declines linearly during the course of dyspneic stimulation. Since we did not expect potentiation but rather a reduction in startle, it was important to make sure any reduction in startle wouldn't be due to habituation. Therefore it was important to have a design which would allow us to statistically correct for habituation-bound decrease in startle. For this reason, startles were measured during a baseline phase prior to the stimulus phase, and during a recovery phase following the stimulus phase, so that a best fit line could be calculated which would filter out the effects of habituation. Another new element in the current experiment was that respiratory loads were applied both during inspiration and expiration, so that startle eliciting probes would always be administered during actual stimulation.

To test the general conclusions we made earlier, we respectively expected to observe:

(a) A reduced startle blink magnitude during aversive interoceptive stimulation, as compared to prior and following an aversive interoceptive stimulus. Given our design, this would correspond to a reduction of startle during stimulus phase as compared to baseline and recovery phase.

(b) For both dyspneic stimuli, we hypothesized a progressive reduction of the startle magnitude during the stimulus phase, as unpleasant dyspneic stimuli have been shown earlier to be associated with reduced startle responding, and as these stimuli are thought to become progressively more unpleasant as time since onset increases.

(c) For the CPT, we expected a quadratic response pattern during the stimulus phase, given that the overall average of multiple startle responses is associated with a reduction in amplitude (Tavernor et al., 2000) while no such reduction has been evident during the 30 to 60 seconds period following stimulus onset (De Peuter et al., 2009), the latter which is perhaps due to the fluctuations in pain sensations during cold stimulation.

In line with earlier findings, it was expected that all stimuli would be scored as unpleasant rather than pleasant, that these stimuli would induce some self-perceived arousal as opposed to complete calmth, lead to sub-maximal levels of feelings of dominance, and to induce some fear.

2. Materials and Methods

2.1. Participants

Thirty-six female psychology freshmen (mean age: 19y/old) participated in return for course credit. Exclusion criteria were pregnancy, presence or history of cardiovascular disease, pain-related conditions, or respiratory disease. Participants were randomly assigned to one of six orders of stimulus presentation – stimulus presentation orders were counterbalanced. The study protocol was approved by the Ethics Committee of the Department of Psychology in accordance with the Declaration of Helsinki (I. World Medical Association, 1997); prior to participation, all subjects read and signed an informed consent with information about the sensations that could possibly follow from exposure to the stimuli, a guarantee about anonymity, and that participation was voluntary and could be terminated at any point in time without loss of the promised course credit.

2.2. Stimuli and Apparatuses

2.2.1. Cold Pressor

The Cold Pressor Test (CPT) was used as a cold pain (CP) stimulus. The CPT consisted of a Plexiglas water basin (Julabo®, Seelbach, Germany), model 19A, containing a type FT200 cooler and type ED water circulator. During the CPT, participants were requested to immerse their right hand to the wrist in this water-filled basin positioned on the right-hand side of their seat. The water with a constant temperature of 6°C was circulated to prevent buildup of warmer water around the hand; the hand was to be held in the cold water for a duration of 90 seconds. Pain at this temperature is experienced as intense, very cold and deep, and produces sympathetic autonomous responses (Casey, Minoshima,

Morrow, & Koeppe, 1996). Participants were explicitly told beforehand that this was not a pain tolerance test and were informed about the duration the hand had to be held in the cold water; this information was provided with the purpose of discouraging participants to withdraw their hand prematurely, although they were free to withdraw their hand at any time. In the 90 seconds prior to and the 90 seconds following the CPT, participants immersed their hand in a stainless steel water basin, model FBATH18 (Techne®, Staffordshire, United Kingdom), with the water having a constant temperature of 30°C and circulating by means of the TE-10D Tempette® thermo regulator and circulator. Immersing the hand in water of 30°C prior and following the CPT was intended to create equal conditions for everyone during the experiment. The two approximately equally sized water basins –one cold, one lukewarm – were purposefully chosen for their visual distinctiveness as to prevent subjects from immersing their hand in the wrong basin at the wrong time.

2.2.2. CO_2

A gas mixture of CO_2 enriched air, with a proportion of 7.5% CO_2, 21%O_2 and 71.5% N_2 was administered for a duration of 90 seconds to induce sensations of dyspnea. The decompressed gas mixture was contained in a meteorological balloon and connected to the inspiratory port. Apart from dyspneic sensations and altered respiratory behavior, 7.5% CO_2 enriched air can elicit (transient) sweating, feelings of warmth, and dizziness (Devriese, De Peuter, Van Diest, Van de Woestijne, & Van den Bergh, 2006; Stegen et al., 1998). Effects of CO_2 inhalation are thought to be cumulative, with less effect on the first few breaths. Similarly, after termination of CO_2 administration, the blood pH level gradually –not instantaneously– returns to its normal level, an effect which is referred to as washout.

2.2.3. Resistive loads

Resistive loads require extra effort from the respiratory muscles – the diaphragm and intercostals – during breathing, in order to maintain flow rate and volume. The accompanying sensation is comparable to breathing through a narrow tube and resembles dyspneic sensations experienced in COPD, asthma and other types of obstructed breathing (Younes, 1995). Unlike CO_2 administration, loaded breathing can be noticed from the first breath. Prolonged loaded breathing may additionally have some cumulative effects, as respiratory muscles can become fatigued. In the current study, two resistive loads were used: one was applied to the inspiratory valve, and one to the expiratory valve. Applying both an inspiratory and expiratory load on breathing ensured that stimulation was continuously unpleasant as it was for the other two stimuli, and that the startle eliciting probe would always fall during actual stimulation. Both loads were of an intensity of 1.96 kPa l^{-1} s, an intensity rated as unpleasant (Pappens et al., 2010).

2.2.4. Breathing apparatus

Throughout the experiment –except during self-report – participants breathed through a mouthpiece while wearing a nose clip. The mouthpiece was fitted on a microbial filter, which in its turn was connected to a non-rebreathing valve to ensure that inspiratory and expiratory air remained separated. The inspiratory and expiratory port of the non-rebreathing valve were both connected to a manual directional control three-way T-shape™ stopcock-type™ valve (Hans Rudolph, inc., series 2110) by means of a vinyl tube (inner diameter 3.5cm; length 100cm). In the loaded breathing trial, the valves allowed easy switching between loaded and unloaded breathing. In the CO_2 inhalation trial, the three-way valve allowed easy switching between breathing room air and CO_2 enriched air on the inspiratory side.

2.2.5. Eye blink startle response

Electromyographic (EMG) activity of the left orbicularis oculi muscle as response to acoustic startle probes (95dB) was measured by the placement of three electrodes filled with high conductivity Microlyte electrolyte gel. One electrode was placed perpendicular under the pupil when the eye was in forward gaze, the second approximately 1 to 2 cm lateral to the first (center-to-center) following the curvature of the eye, and one signal ground electrode was placed on the center of the forehead. All sites were first cleaned with alcohol to reduce inter-electrode resistance. The raw signal was amplified by a Coulbourn isolated bioamplifier with bandpass filter (LabLinc v75-04) with a 90Hz high pass filter. This signal was routed to a Coulbourn 4 channel integrator (LabLinc v76-24), which rectified and smoothed the signal online with a time constant of 20ms. The EMG signal was sampled at 1000Hz starting 500 ms prior to the onset of the auditory startle probe, until 1000ms after probe onset.

2.2.6. Software

All signals were transmitted through a 16-Bit National Instruments PCI-6221 data acquisition card (National Instruments, Austin, Texas) to a computer. Affect 4.0 software (Spruyt et al., 2010) was used for running the experiment as well as for data acquisition. A modular script-based program named PSychoPHysiological Analysis and abbreviated as PSPHA (De Clercq et al., 2006) was used to handle the recorded signals offline and to extract the relevant parameters necessary for statistical analysis.

2.2.7. Self-report measures

At the end of each trial a computerized 9-point scale of the language-free Self-Assessment Manikin (SAM-Scale, Bradley & Lang, 1994) was administered to retrospectively rate the mean valence (unpleasant = 1; pleasant = 9), arousal (calm = 1; excited = 9), and dominance (lack of control = 1, sense of control = 9) felt during the 90 second stimulus. Before proceeding to the next trial, subjects were requested to indicate their fear as experienced during the stimulus period on a computerized horizontal Visual Analogue Scale (VAS; 0 = not at all scared; 100 = extremely scared).

2.3. Procedure

Upon arrival, the experimenter led the participants into the experimental room where he provided them an informed consent. The informed consent briefly mentioned all stimuli, as well as the sensations each stimulus may respectively elicit, and that any sensations felt were without harm and were of a transient nature. Participants were requested to read through the consent before agreeing to sign it. After signing, a brief questionnaire of medical history in relation to exclusion criteria (see 2.1) was provided. Next, electrodes were attached – subjects were informed these were meant for measuring physiological responses, albeit without further specifications. The experimenter verbally went through the experimental procedure, and then placed headphones on the participant. Participants were requested to put on the nose clip and breathe through the mouthpiece, and told to keep their eyes fixated in the direction of the computer screen. Participants sat upright (not reclined) throughout the entire experiment and lights remained on (not dimmed). Prior to initiation of the experimental manipulations, the experimenter left and went to the operator room, adjacent to the room where participants were left alone throughout the entire experiment; the experimenter remained in the operator room until the experiment was over.

Prior to each trial, 10 acoustic startle probes were administered to habituate participants to the startle probe. Habituation to the probes was done because startle responses tend to be amplified at initial presentation of the acoustic probes due to their novelty. During habituation the interval between probes varied from 19 to 21 seconds. After startle probe habituation, a trial started off with a baseline phase of 90 seconds where subjects breathed through a mouthpiece, fixated their eyes on a computer screen, and received three startle probes at unpredictable times, one during the first, one during the second and one during the last 30 seconds. Additionally, in the CPT trial, subjects immersed their hand in lukewarm water during baseline. The second phase was the stimulus phase during which either of the three stimuli – cold water, CO_2-enriched air, or loaded breathing – was administered. During this phase, again there were three startle probes at variable times – one during the first 30 seconds, one during the second, and one during the last. The third phase is referred to as the recovery phase, and was identical to the baseline phase, except in that it followed –instead of preceded – the stimulus phase. After recovery, subjects were free to release the mouthpiece while they filled out the self-report scales, before proceeding to the next trial. In total there were three trials, and only one (continuous) stimulus was presented per trial, during the stimulus phase. Avoiding repeated presentation of stimuli ensured that potential learning behavior and alteration of responses due to recent exposure was minimized. At the end of the experiment, subjects were fully debriefed.

2.4. Data analysis

Eye blink startle EMG responses were calculated by subtracting the mean value from the 0 to 20 ms following probe onset from the peak value found in the 21 to 175ms time window following probe onset. Excluding startle measured during habituation, there were 27 remaining data points per subject. Data points where there was already blink activity at the onset of startle probe presentation were rejected (<10%) as recommended by Blumenthal et al. (2005). The maximum percentage of missing data points for a single subject was just under 26%, while the mean number of missing data points per person was just over 7%. After removing rejected values, startle amplitudes were transformed to T-scores for each individual, which is a common procedure (see Blumenthal et al., 2005). The reason for this transformation was that we were interested in overall intra-individual differences in response to the different phases and probe delivery times, and not in inter-individual differences in response amplitude. Having obtained individual T-scores, missing data were replaced by the mean startle amplitude in response to the same probe of those people who had received all stimuli in the same order. We did this to rule out effects of stimulus presentation order on amplitude. Once missing data were replaced, the data were detrended by using individual regression models with probe order as the predictor (Lüthy et al., 2003). Using this method, the mean of a best fit line was subtracted from the actual T-score. Unless this method is applied, magnitudes in our design are generally higher during baseline, and lower during recovery, simply because startle magnitudes continue to decline linearly, even after initial habituation. By removing this linear reduction in magnitude, the magnitudes at each point in time across the three phases become better comparable, and the differences in amplitude that remain are more likely due to the sensations at that moment of prolonged stimulation.

The detrended data were then entered into repeated measures ANOVA's, with TRIAL TYPE (CPT, CO_2 or load trial), PHASE (baseline, stimulus, or recovery), and STARTLE PROBE TIMING (1^{st} 30s, 2^{nd} 30 s, or last 30s) as within subject variables. In order to test our first hypothesis that startle would be reduced during aversive interoceptive stimulation as compared to baseline and recovery phase, we performed a polynomial quadratic contrast for the effect of phase on all data points. In order to test our second hypothesis that startle would progressively decrease during prolonged dyspneic stimulation, we performed a polynomial linear contrast on the effect of startle probe timing on the data points obtained during the stimulus phase of both dyspneic stimuli. And finally, in order to test our third hypothesis that startle in response to the CPT would show a reduction in amplitude overall, except during the 30 to 60 seconds following onset, we performed a polynomial quadratic contrast on the data points obtained during the stimulus phase of CPT.

The SAM scores for valence of the three stimuli were compared using a one-way repeated measures ANOVA. The same analysis was done for the SAM scores on arousal as well as for dominance levels and VAS state anxiety scores compared in response to the three stimuli.

An α-level of .05 was set for statistical significance. Analyses were done using the STATISTICA Version 10 software package and the means and standard deviations displayed in Table 3 were obtained using the JMP 9 software package.

3. Results

3.1. Eye blink EMG

To test our first hypothesis that startle is reduced during interoceptive stimulation (all three stimuli), a univariate test of significance for planned comparisons of least square means for the effect of PHASE confirmed the existence of a quadratic contrast, $F(1, 35) = 6.19$, $p < .05$, meaning that startle dropped from baseline phase to stimulus phase, and rose again from stimulus phase to recovery phase (see figure 4a). To test our second hypothesis that dyspneic stimuli lead to a progressive decrease in startle responding, another test for planned comparisons was performed for the effect of STARTLE PROBE TIMING during stimulus phase of both dyspneic stimuli, and found a linear decrease in startle magnitude, $F(1, 35) = 4.20$, $p < .05$ (see figure 4b). In contrast, the effect of STARTLE PROBE TIMING during the stimulus phase of the CPT displayed a quadratic pattern, $F(1, 35) = 4.68$, $p < .05$. That is, during the stimulus phase where the CPT was administered, there was an initial reduction in startle amplitude, followed by an increase in amplitude, which was in its turn followed by a decrease in amplitude again (see figure 4c).

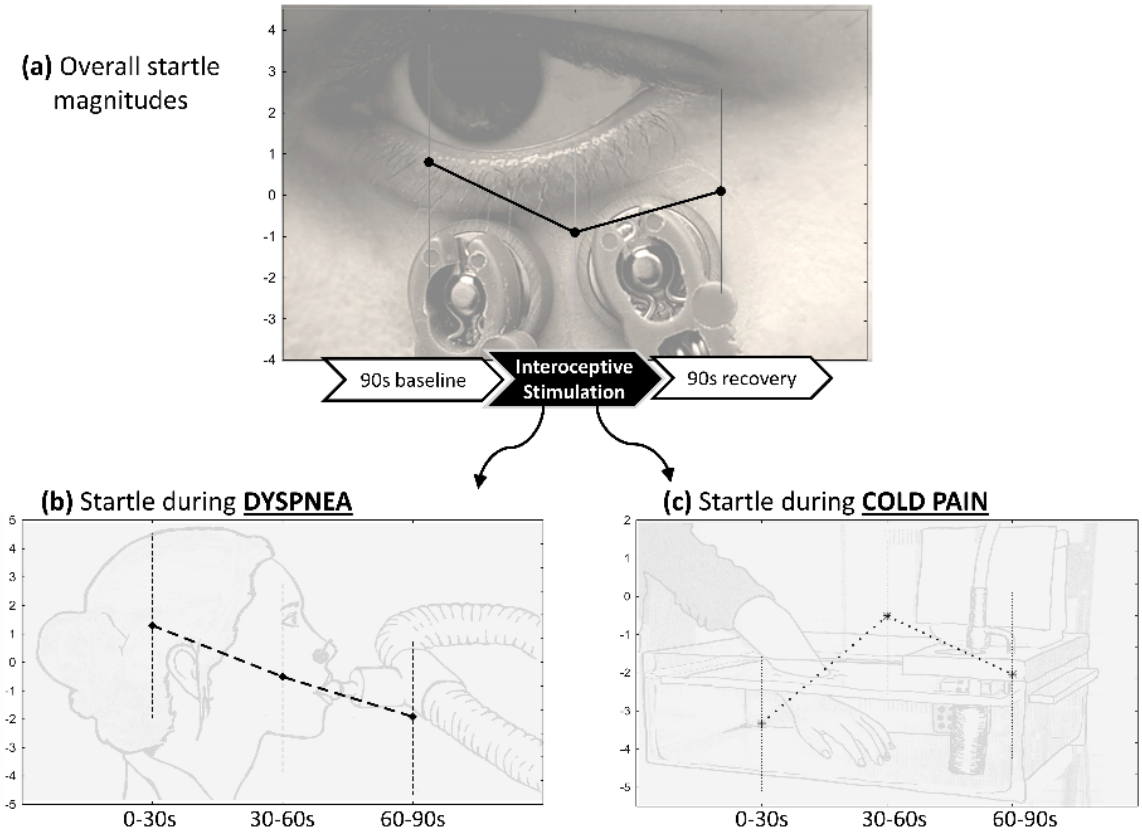

Figure 4. Graphs showing detrended startle magnitudes per phase **(a)**, and of individual startles during stimulus phase only for respiratory stimuli **(b)** and for peripheral hypothermia **(c)**. Values are presented as magnitude means $\pm$ 0,95 confidence intervals.

3.2. Self-report

As evident from Table 4, the three stimuli evoked similar levels of arousal, dominance, and fear. The only significant difference between the stimuli was in perceived valence, $F(2, 70) = 5.52$, $p < .01$, with Tukey-Kramer post-hoc tests indicating that CP was rated as more unpleasant than both CO_2 inhalation ($p = .01$) and loaded breathing ($p < .05$).

Table 4

Means and standard deviations for valence, arousal, dominance, and fear experienced during stimulation

	cold pain		CO_2 inhalation		loaded breathing	
	MEAN	SD	MEAN	SD	MEAN	SD
valence	3_b	1.6	3.8_a	1.5	3.8_a	1.6
arousal	6_a	1.9	5.6_a	1.7	5.4_a	1.8
dominance	3.9_a	1,9	4.4_a	1,9	4.5_a	2.2
fear	43_a	24	47_a	25	45_a	30

Note. Valence, arousal, and dominance all ranged from 1 to 9, respectively unpleasant versus pleasant, calm versus excited, and a lack of control versus a sense of control. Fear ranged from 0 to 100, respectively from not at all scared to extremely scared. Means in the same row which share a subscript are not significantly different from one another according to Tukey-Kramer post-hoc tests.

4. Discussion

The current study had as aim to elucidate the startle response pattern during aversive interoceptive stimulation, and used a sample of 36 young adult females to do so. To date affective modulation of startle has almost exclusively been studied using visual and auditory stimuli, mental imagery, and sometimes using *anticipation of* aversive interoceptive sensations (e.g., Lang et al., 2011; Melzig et al., 2008). However, reports on startle *during* aversive interoceptive stimulation are still very scarce. In this initial study on within-subject responses to different types of interoceptive stimulation, two respiratory stimuli commonly used in studies on dyspnea and fear, and one cold stimulus commonly used in research on pain were presented to participants. In order to effectively document startle not as a conditioned response, but as an unconditioned response, all stimuli were administered only once,

which necessitated they be administered for a relatively prolonged duration in order to allow for the administration of multiple startle probes.

The findings from our current study are in accordance with the scarce amount of previously published data, in that startle responding to these three interoceptive stimuli is reduced overall. A new insight from the current study is the presence of a linear decrease of startle responding in face of dyspneic stimulation, and a non-linear, quadratic startle response pattern during the CPT.

The overall reduction of startle for all three stimuli is evident despite that all three stimuli are rated as fearful, unpleasant, arousing, and associated with sub-maximal levels of dominance. Although the ratings of unpleasantness are not at the extreme end of the valence-scale, an earlier study of Pappens (2010) found that the intensity of respiratory loaded breathing which we also used in the current study, was more aversive than aversive pictures from the International Affective Picture System (IAPS). Moreover, the CO_2 inhalation in the current study was equally aversive as loaded breathing, and CP was even more aversive. That participants in the current study refrained from filling in the extremes of the valence and fear scales does not indicate the stimuli were ineffective in inducing unpleasantness or fear. Rather, we argue this underreport to be due to the lack of milder unpleasant stimuli (e.g., unpleasant pictures), and the anticipation that a potentially more aversive stimulus might be presented in a subsequent trial (requiring that the extremes of the scale need to remain unused until then). Though we did not let subjects rate the baseline and recovery phases, right after the experiment was over subjects informally informed the experimenter that those phases were dull (they had to stare at a fixation cross and knew no stimuli would be presented during those phases), thus ruling out that the affective tone was constantly negative.

From the perspective of the emotional priming model, which posits that startle magnitude should be greater when the aversive motivational system is active (Lang et al., 1998), the overall reduction in startle is puzzling. Although the few previous studies that found similar results forwarded a number of explanations, currently there is no satisfactory answer to the mechanisms underlying this unusual response pattern. Nevertheless, based on prior explanatory speculations, some suggestions for future research can be made.

One speculation that has been made earlier, is that the reduction in startle responding is due to the interoceptive nature of the stimuli (Pappens et al., 2010), implying that aversive interoceptive stimulation of any kind would fail to evoke potentiation. Although the current study did not find any counterevidence for this claim, further research with other types of interoceptive stimulation is necessary in order to truly falsify this claim. Moreover, resorting to the interoceptive nature of the stimuli as an explanation for the unusual startle response, requires a predefined and well-outlined

working definition of interoception, given that consensus on its definition is lacking, in particular with regards to the 'outer boundaries' of the concept (Dworkin, 2007b).

Another explanation forwarded by Pappens et al. (2010) is that according to the defense cascade model (Lang et al., 1997), startle potentiation should no longer be evident during the circastrike (fight/flight) phase of defensive responding, despite averseness of stimulation (e.g., Low, Lang, Smith, & Bradley, 2008; Richter, Hamm, Pané-Farré, Gerlach, Gloster, Wittchen, Lang, Alpers, Helbig-Lang, Deckert, et al., 2012). This circastrike phase of defensive responding is distinct from earlier defensive phases, not only in startle responding and threat imminence, but also in autonomous responses such as heart rate, skin conductance, and presumably in respiration as well (Van Diest, Bradley, Guerra, Van den Bergh, & Lang, 2009). In order to test whether the startle response pattern in face of interoceptive stimuli might be due to activation of circastrike responding, inclusion of additional autonomous measures could theoretically provide further conclusive evidence. In practice however, many interoceptive stimuli, including the stimuli used in this experiment, elicit regulatory homeostatic responses, which may complicate interpretation of autonomous measures, making this hypothesis hard to test for at least a number of interoceptive stimuli.

Finally, orientation of attention to bodily processes has been speculated to be responsible for a reduction in responsiveness to auditory stimuli such as the startle probe (Pappens, De Peuter, et al., 2012). This speculation could be tested by manipulating orientation of attention to bodily processes or to surrounding stimuli such as acoustic probes. To date, only one such study has been done and suggests orientation of attention inward may be responsible for a reduction in startle responding to respiratory loads (M. Pappens et al., 2011a), but it remains unclear whether this could also explain startle in response to CO_2 or the CPT. An alternative method to corroborate this explanation, is to include a measure of attention requiring subjects to indicate whether their attention was oriented predominantly at bodily sensations, predominantly at surrounding stimuli, or divided between both.

In the current study, these explanatory hypotheses were not extensively put to the test, as the primary aim was to describe, not explain the response pattern to the interoceptive stimuli we selected. Nevertheless, the present findings provide sufficient reason for taking these hypotheses and the methods to test them into account in future studies. Outlining the definition of interoception, testing startle in response to other forms of aversive interoceptive stimulation, inclusion of other psychophysiological measures in some instances, and manipulation and/or measures of orientation of attention are all potential avenues for future research, which may elucidate the mechanism responsible for the atypical startle patterns observed in the current and previous studies. Additionally, possible sex differences in the subjective experience and/or in the psychophysiological response pattern may require more attention in future studies, given that psychosomatic complaints are

predominantly present in women (Kroenke & Spitzer, 1998; Şar, 2010). Until these issues are addressed in further studies, any explanatory hypotheses remain speculative at best. For now, we are left with only a descriptive model of startle to aversive interoceptive stimulation.

In this respect, it needs mention that the startle-by-startle analysis, a method usually rejected in favor of averaging magnitudes of startles delivered at different times, may actually provide additional insight into the pattern of responding over the course of time. The startle-by-startle analysis accounts for discrepancies between the study of Tavernor (2000) and an earlier study of ourselves (De Peuter et al., 2009); our current findings illustrate that although startle responding may be generally reduced following CPT, it is not necessarily reduced at all points in time following onset of this stimulus. The magnitude increase during the 30 to 60 seconds interval that we have found a second time now, warrants a startle-by-startle analysis in addition to the more common averaging method, especially when startles are administered during prolonged aversive stimulation. Moreover, further research on the CPT and its concomitant fluctuations of sensory discomfort over the course of time are necessary, as these sensory fluctuations may underlie the fluctuations in startle responding. Currently, such research is very limited (e.g., Davis & Pope, 2002).

In conclusion, the evidence for an unusual startle response pattern during interoceptive stimulation is becoming more substantial. Although it is commonly assumed that startle is potentiated during aversive emotional states including fear, an opposite pattern has been found for a number of fearful interoceptive stimuli. A startle-by-startle analysis suggests this to be dependent on subjective fearfulness which generally increases following onset of respiratory stimulation, but presumably fluctuates for CP induced by the CPT. Further research is needed to test this hypothesis more thoroughly, and to find out if the results are specific to women, or whether they also apply to men.

CHAPTER 6

Visceral pain modulates startle differently in men and women

Ceunen, Zaman, Herssens, Van Oudenhove, Bogaerts, Ly, Coen, Tack, Vlaeyen, Van Diest (submitted). *International Journal of Psychophysiology*

Abstract:

This study aimed to investigate affective modulation of eye blink startle by aversive visceral stimulation. Startle blink EMG responses were measured in 31 healthy participants receiving painful, intermittent balloon distentions in the distal esophagus during 4 blocks (positive, negative, neutral or no pictures), and compared to startles during 3 'safe' blocks without esophageal stimulations (positive, negative or neutral emotional pictures). Women showed a fear-potentiated startle potentiation during blocks with distentions, both when the balloon was in an inflated and deflated state. Men tended to show a reduced startle blink during actual inflation of the esophageal balloon. Affective picture viewing did not modulate the startle blink. The present findings corroborate earlier findings that women and men differentially recruit attentional and fear networks during aversive visceral stimulation.

1. Introduction

Eye blink startle is modulated by affective background (Vrana et al., 1988). As such, it can be used to distinguish between appetitive, neutral, and aversive emotional states, with decreased magnitudes during the former, and increased magnitudes during the latter. This affective modulation of startle is only evident when the emotional states are sufficiently arousing or intense (Bradley et al., 2001). Such emotional and physiological arousal can be indexed by skin conductance, reflecting sympathetic activation (Dawson, Schell, & Filion, 2007). Together, eye blink startle magnitude and electro-dermal measures can be used to assess the biphasic aspects of emotion, one reflecting motivational direction, and the other motivational intensity (Bradley & Lang, 2007).

Because affective modulation of startle has been found when using visual (e.g., Jansen & Frijda, 1994; Schupp et al., 1997), auditory (Bradley & Lang, 2000), and olfactory (Ehrlichman et al., 1995) mood stimuli, it has been suggested that such modulation occurs regardless of the sensory modality used for mood induction (Bradley & Lang, 2007). Recent research with interoceptive stimuli seems to contest this notion (Ceunen et al., 2013). For example, during aversive and arousing dyspnea as induced by loaded breathing (a mechanical stimulus creating respiratory resistance similar to breathing through a straw), startle potentiation has not been evidenced (Pappens et al., 2010). Moreover, when fear-inducing dyspnea was elicited by CO_2 inhalation, it led to inhibition of startle, relative to startle measured during room air breathing (Pappens, De Peuter, et al., 2012). Also during cold pain, which is interoceptive according to the definition of interoception forwarded by Craig (2002), no startle potentiation was observed (Deuter et al., 2012). In contrast, during anxious *anticipation* of respiratory and other interoceptive sensations, the expected startle potentiation has been found (Hubbard et al., 2011; Lang et al., 2011; Melzig et al., 2008; B. Naliboff et al., 2009; Pappens et al., 2013; Twiss et al., 2009).

Apart from a small number of studies, at present the pattern of startle in response to emotions induced by actual presence, rather than anticipation of interoceptive sensations (including pain), largely remains to be elucidated. Therefore, the major aim of the current study was to unveil the startle response pattern that occurs in a period of time during which there is repeated exposure to an aversive interoceptive stimulus, namely stimulation of the distal esophagus at pain threshold, i.e. first sensation of pain. We hypothesized that startle potentiation would occur during 'unsafe' periods during which painful stimulation could occur relative to 'safe' periods without such stimulation.

An additional aim was to find out whether startle potentiation is present both during anticipation of and during actual painful stimulation, relative to safe periods. Based on the various findings on startle in response to interoceptive stimuli as discussed earlier, it would be expected that

startle in anticipation of visceral stimulation is elevated relative to startle elicited during actual visceral stimulation.

Moreover, as findings in the literature suggest that sex differences exist in neurobiological mechanisms involved in the processing of visceral signals (Kano et al., 2013; Kilpatrick et al., 2010; Labus et al., 2013; Pennebaker & Roberts, 1992), we also aimed to explore whether gender modulated the startle response patterns in relation to the painful esophageal stimulus. However, as the literature on startle in response to interoceptive stimuli is on itself already relatively limited, it follows that the literature on gender effects on startle during interoceptive stimulation is nearly non-existent. Therefore we did not make any specific assumptions on how the startle would be different between genders, if at all, even if there are clear indications for the existence of gender specific differences in the processing of interoceptive stimuli.

The choice for distal esophageal stimulation was in part motivated by the ability to stimulate solely visceral tissue without involving stimulation of any overlying somatic tissue (Aziz et al., 2000), thus being classified as an interoceptive stimulation even by those who define interoception in its strictest sense (e.g., Dworkin, 2007). Opting for the esophagus as the site of stimulation also allows for future research to expand upon the current research findings, for example contrasting purely visceral stimulation (distal esophagus) with purely somatic stimulation (proximal esophagus) (Aziz et al., 2000). We decided to stimulate at first pain threshold for our stimulus to qualify as aversive; pain by definition comprises a component of unpleasant affect according to the International Association for the Study of Pain (Merskey & Bogduk, 1994).

Given the extensive literature of affective modulation of pain (Rhudy & Meagher, 2001; Wiech & Tracey, 2009), we included three different affective backgrounds by means of pictures. These were included in order to explore whether these backgrounds would differently affect the eye blink startles elicited during blocks with esophageal distention compared to those without. Inclusion of the emotional picture series also controlled for any extraneous confounding factors that might affect mood of participants.

Last, we also included one block during which esophageal distentions were administered without any background pictures. This condition allowed us to explore whether visceral stimulation with concurrent picture viewing affected startle differently than stimulation without picture viewing.

2. Methods

We recruited 31 healthy university students; they received 50 € for participating in the study. As 7 participants were excluded from analysis (see Data analysis section, subheading Eye blink startle) we only studied the responses of the remaining 24 participants (14 women); they had a mean age of 22

years old (SD = 3). All participants received an informed consent prior to deciding on whether to participate, and reread the consent just prior to signing it. The informed consent was in accordance with the declaration of Helsinki (1997) and stated that participants were free to halt their participation at any point without any negative consequences. This study was approved by the psychological and medical ethical committees of KU Leuven.

At the start of the experiment, a standard pediatric catheter was inserted trans-nasally with the end reaching the distal, autonomously innervated part of the esophagus, 35cm from the nostril. A deflated medical balloon was firmly attached to the end positioned in the esophagus, while the extraneous part of the catheter was gently attached to the face with tape to prevent it from moving. The remaining end was draped over the ear and connected to an air filled syringe. Although the insertion itself was invariably experienced as unpleasant, once the catheter was in its proper position, we did not continue with the next steps of the procedure, until subjects reported they became habituated to any sensations due to the presence of the catheter, which never took more than a few minutes. This procedure has been used extensively in previous research (e.g., Aziz et al., 2000; Coen et al., 2009).

After inserting the catheter, the pain threshold of participants was determined by gradually inflating the esophageal balloon thrice, and taking the average of these three volumes of distention (at which subjects indicated they first felt a sensation that they would call painful) as the best approximation of their actual pain threshold. Additionally, during threshold determination, we assured ourselves that the balloon was in the distal part of the esophagus by asking participants if they could indicate where they felt a sensation: if their answer indicated they could feel the sensation somewhere around their chest level, but that they could not locate it at a specific site, this was taken to indicate the balloon was indeed in the autonomously innervated, i.e. visceral part of the esophagus (Aziz et al., 2000). After threshold determination, a 3-minute baseline measure of skin conductance was obtained, and subjects were exposed to 10 startle probes in order to habituate them before proceeding to the actual experiment. There was an interval of 10 seconds between each of these habituation probes.

The experiment consisted of seven blocks lasting 5m23s each. In six of the seven blocks, participants viewed a series of 36 mood-inducing pictures of one same valence, selected from the International Affective Picture System (IAPS, Lang, Bradley, & Cuthbert, 2008). Each picture was presented only once throughout the entire experiment (8s on, 1s off). Two picture blocks contained positive pictures, two blocks contained neutral pictures, and two blocks contained negative pictures. Both blocks of each valence had equal mean valence, arousal, and dominance levels according to the normative data collected by Mikels et al. (2005). Furthermore blocks of the same valence were also

matched according to the proportion of animals, objects, humans, and overall picture content complexity (see appendix for more information of the exact pictures that were selected). A similar blocked presentation of pictures has been used earlier by Smith, Bradley and Lang (2005), and results of their study indicate that affective modulation is maintained and even increased throughout the consecutive presentation of pictures of the same valence.

In the block without pictures, participants were instructed to look at a fixation cross presented on the monitor. The latter block, as well as 3 of the picture viewing blocks (one for each picture valence) were accompanied by 10 esophageal balloon distentions. Balloon distentions in those blocks were administered manually at individual pain threshold, started simultaneously with picture onset (in the distention blocks with pictures), and ended after 5 seconds. Inflations and deflations were performed as instantaneous as physically possible, implying that throughout each distention, the balloon's volume was nearly constant. The available air volume for inflation was limited to the individual threshold to prevent accidentally exceeding the determined volume. The order of the block presentations was semi-randomized, taking into account that blocks of the same pictorial valence or blocks with distentions would never be presented consecutively. The first, third, fifth and seventh blocks were blocks with distentions, whereas the second, fourth and sixth block were free of distentions. Participants were informed that blocks with and blocks without distentions would alternate, and were informed that a new block would start only after filling in self-report items. The 10 distentions in each of the four distention blocks occurred with varying intervals between each distention (22-40s), making the exact onset of each distention unpredictable.

Self-reports of fear, valence and arousal were obtained after each block, respectively on a horizontal VAS ranging from 0 (no fear at all) to 10 (worst fear imaginable), and two 9-point self-assessment manikin (SAM; Bradley & Lang, 1994) scales, one ranging from unpleasant (1) to neutral (5) to pleasant (9), and the other from calm (1) to aroused (9). Furthermore, participants rated pain intensity on vertical bars after each block. Scores ranged from 'no sensation' (0) to 'moderate' (5) to 'most intense I can imagine' (10). Finally, we also assessed to which extent persons had experienced a set of 10 hyperventilation symptoms on a 5 point Likert-scale. The latter self-reports addressed an exploratory research question that goes beyond the scope of the present paper and will not be further discussed here.

Per block, 10 white noise startle probes (50ms) with a peak dBA of 103dB were administered binaurally. Although startles were always administered 4 seconds after picture onset, their occurrence was made unpredictable by varying the interval between startle probes (27-54s). The EMG eye blink startle responses were measured by three Ag/AgCl Coulbourn electrodes (V91-02, 4mm) according to the guidelines described by Blumenthal et al. (2005). A V75-04 (Coulbourn Instruments) isolated

bioamplifier with a 13Hz high pass, and 1 kHz low pass bandpass filter was used to amplify the raw signal. This signal was then rectified and smoothed by a Coulbourn integrator (V76-24; time constant=20ms). Startle responses were sampled at 1000Hz and were recorded starting 500ms prior to probe onset until 1000ms after probe onset.

Skin conductance levels (SCLs) at the hypothenar eminence of the non-dominant hand were recorded throughout each block using a Coulbourn skin conductance coupler (V71-23) providing a constant of 0.5V across two V91-01 (8mm) electrodes. The signal was digitized at 10Hz.

Affect 4.0 software (Spruyt et al., 2010) and a 16-bit data acquisition card (National Instruments, Austin, Texas) were used to collect EMG and SCL data. Physiological data were organized using JMP® 9 software, further processed offline for parameter extraction with PSPHA (De Clercq et al., 2006), and analyzed using STATISTICA 10 software.

3. Data Analysis

3.1 Manipulation check

Pain ratings of all blocks were entered in a repeated measures ANOVA with gender as a between subject variable and block as a within subject variable (7 levels). An a priori assumption was that pain intensity would be higher during distention trials as compared to non-distention trials for both genders. To explore potential sensitization or habituation effects on pain, we also ran a repeated measures ANOVA with position of the distention block (1st, 3rd, 5th or 7th) as a within subject variable and gender as a between subject variable.

3.2 Eye blink startle.

Eye blink EMG magnitudes were obtained by subtracting the mean baseline value (0 to 20ms following probe onset) from the peak value (21 to 175ms following probe onset). All startle responses were visually inspected and values were discarded when there was already blink activity between startle probe onset and minimal blink onset latency; this was the case in 16.9 % of the cases. Four participants showed no or rejected startle response in > 66% of all startle trials; those participants were classified as non-responders and further excluded from analyses (Blumenthal et al., 2005).

Within the distention blocks and irrespective of picture viewing, another 3 participants failed to show at least 4 valid startle responses either during actual balloon inflations, either in between distentions (deflations). In order to avoid that unreliable estimations of the startle response (based on less than 4 startles during actual inflation or deflation) would affect our findings, these participants were also excluded from analyses, resulting in a final sample of N = 24 (14 women).

We averaged each participant's startle responses for 11 within subject conditions (see also Table 5). Three conditions (further called 'safe') referred to distention-free blocks of picture viewing

(one positive, one neutral, one negative). Four other conditions (further called 'inflation') referred to startles during actual distention (positive, neutral, negative pictures, and no pictures). The last four conditions (further called 'deflation') were blocks with distentions (positive, neutral, negative pictures, and no pictures) in which the startle probes were delivered at times the balloon was in a deflated state. Next, the averaged raw startle magnitudes for each of these 11 conditions were standardized within participants (T-scores), so that they would be relative to each subjects' range of responding. Also for the 8 inflation and deflation conditions of the distention blocks, data of cells that were based on less than 4 valid startle responses, were set as 'missing data'. This criterion was set to ensure reliable startle estimates for each of the eight inflation and deflation condition, but resulted in 27.7% empty cells in our data matrix. Because of the missing cells, interactions of inflation/deflation with picture content (neutral/positive/negative) were not included in any of the tested models. In other words, effects of picture content in the distention blocks were tested only across inflation and deflation conditions, and effects of inflation/deflation were studied irrespective of picture content. Missing cells were not replaced with data from other cells. Startle responses were analyzed using three mixed regression models testing each a particular research question.

Table 5. *Overview of the 11 within subject conditions.*

Pain	**Pictures**	**Block**		**Condition**
'Safe' (distention-free blocks)	Positive	1		1
	Neutral	2		2
	Negative	3		3
Blocks with distentions	Positive	4	Deflation	4
			Inflation	5
	Neutral	5	Deflation	6
			Inflation	7
	Negative	6	Deflation	8
			Inflation	9
	No pictures	7	Deflation	10
			Inflation	11

A first model tested whether startles during distention blocks differed depending on whether pictures were concurrently presented or not. Data of the safe blocks were not included, as no distensions were presented. The model consisted of startle amplitudes of the 4 distention blocks (positive, neutral, negative or no pictures) as the dependent variable. Predictors were fixed effects for Gender (0 = male/1= female), Picture (1 = picture/ 0 = fixation cross) and a Picture*Gender interaction term. A repeated measures random effect was included to account for the interdependence of data points within one subject. The most general covariance structure that could be fitted was a first order autoregressive structure with heterogeneous variances ($AR1_{(Heterogeneous)}$) (X^2 (7) = 17.16, $p < 0.05$) which had significantly better fit compared to its competitors (Scaled Identity or AR1).

A second model aimed to test whether picture content modulated the startle magnitude, and whether such modulation differed for blocks with and without distention. Data from the fixation cross trials were not included, as no pictures were presented. The model consisted of startle amplitudes of the three different picture blocks (positive, neutral, negative) during safe and distention blocks as the dependent variable. Predictors were fixed effects for Pain (1 = distention / 0 = safe), two dummy variables for picture content (positive (Dum_Pos)/ negative (Dum_Neg)) with neutral pictures as reference coded, Gender (0 = male/ 1 = female) and the following interaction terms; Pain × Dum_Pos, Condition × Dum_Neg and Pain × Gender. In addition, a repeated measures random effect was included and $AR1_{(Heterogeneous)}$ was preferred as covariance structure.

A third model tested whether startles delivered during actual inflation, or during deflation in distention blocks were potentiated relative to the distention-free ('safe') blocks and whether gender influenced this pattern. The model consisted of startle amplitudes for all blocks as dependent variable. Predictors were fixed effects for Gender (0 = male/1 = female), two dummy variables for Deflation and Inflation (Inflated (Dum_Infl)/ Deflated (Dum_Defl)) with the non-distention ('safe') blocks as reference coded. In addition, the following interaction terms were included: Dum_Defl × Gender, Dum_Infl × Gender. Next, a repeated measures random effect was included with $AR1_{(Heterogeneous)}$ as covariance structure.

3.3 Skin Conductance Levels

Mean absolute skin conductance levels (SCLs) were obtained per interval of 9 seconds. These SCLs were corrected for individual differences in response range by means of Rose's range correction (see Lykken & Venables, 1971). Next, a mean was calculated per block for each individual, and then a repeated measures ANOVA with gender as a between subject variable and Block (7 levels) as a within subject variable was performed on the obtained values.

3.4 Evaluative judgments

Self-reported fear, unpleasantness, and arousal scores of each individual for each block were also entered in a repeated measures ANOVA with gender as a between subject variable and Block (7 levels) as a within subject variable was performed. Tukey-Kramer post-hoc tests were used to follow-up main effects of Block.

Greenhouse-Geisser corrections were applied where appropriate; we will report unadjusted degrees of freedom and adjusted *p*-values.

4. Results

An independent samples t-test indicated that thresholds of first pain did not differ significantly between male and female participants with mean distention volumes respectively 19ml (SD = 7ml) and 17ml (SD = 7ml).

4.1 Manipulation check

Following up the main effect of block, F (6, 132) = 126.71, $p < .001$, $\varepsilon = .64$, $\eta_p^2 = .85$, confirmed that participants reported more intense pain for blocks with as compared to blocks without distentions, F (6, 132) = 317.80, $p < .001$. There were no significant main or interactions effects with gender.

The repeated measures ANOVA with position of the distention block as a within subject variable (1st, 3rd, 5th, or 7th) and gender as a between subject variable revealed no significant effects. However, the data showed a trend for women to report higher pain intensities for distention blocks than men (main effect of gender: F (1, 22) = 4.13, $p = .05$, $\eta_p^2 = .16$). Also, the non-significant Gender x Block interaction suggested that men tended to habituate, whereas women tended to sensitize to visceral pain, F(3, 66) = 2.12, $p < .12$, $\varepsilon = .85$, $\eta_p^2 = .09$.

Table 6. *Means (standard deviation) of skin conductance levels, startle magnitudes, and self-reported fear, pain intensity, pleasantness and arousal for each of the seven blocks.*

	Non-distention Blocks			Distention Blocks			
	Pos pic	Neu pic	Neg pic	Pos pic	Neu pic	Neg pic	No pic
SCL	0.42_{ab}	0.46_{ab}	0.39_{a}	0.51_{b}	0.48_{ab}	0.53_{b}	0.52_{b}
	(0.19)	(0.17)	(0.18)	(0.18)	(0.19)	(0.18)	(0.15)
Startle	46.80	48.24	50.01	51.41	51.64	52.86	52.75
	(5.31)	(6.66)	(6.31)	(8.88)	(10.81)	(9.06)	(13.65)
Pain	0.75_{a}	0.71_{a}	0.50_{a}	5.96_{b}	6.25_{b}	6.33_{b}	6.63_{b}
intensity	(1.33)	(1.57)	(1.10)	(1.57)	(1.80)	(1.63)	(1.38)
Fear	0.71_{a}	0.96_{a}	2.58_{b}	2.86_{bc}	3.58_{bc}	3.96_{c}	3.75_{c}
	(0.91)	(1.60)	(2.32)	(2.15)	(2.50)	(2.54)	(2.56)
Pleasantness	6.92_{a}	5.96_{a}	4.25_{b}	4.09_{b}	3.83_{bc}	2.75_{c}	2.88_{c}
	(1.61)	(1.49)	(1.57)	(1.77)	(1.43)	(1.07)	(0.99)
Arousal	3.33_{a}	3.17_{a}	4.33_{b}	4.82_{bc}	5.33_{bc}	5.58_{c}	5.25_{bc}
	(1.83)	(1.37)	(1.83)	(1.46)	(1.83)	(1.53)	(1.59)

Note. Pos pic = positive pictures; Neg pic = negative pictures; Neu pic = neutral pictures; No pic = without pictures; SCL = Range-corrected Skin Conductance Level; Startle = T-score of the Startle Blink Magnitude; Pain intensity (0-10 scale); Fear (0-10 scale); Pleasantness (1=unpleasant; 9=pleasant); Arousal (1=calm; 9=aroused). Means in the same row which share a subscript are not significantly different from one another according to Tukey-Kramer post-hoc tests ($p < .05$).

4.2 Eye blink startle

The mean startle magnitudes for each of the seven blocks can be read from Table 6. Figure 5 displays the mean startle response for men and women during inflation, deflation and during non-distention, irrespective of picture viewing and picture content.

Model 1. Because the interaction between gender and picture was not significant, $F(1, 35.39) = .32$, $p = .58$, it was removed from the model. There was a significant gender effect, $F(1, 42.11) = 5.30$, $p = .03$, with women showing higher startle amplitudes during the distention condition compared to men. Startle responses in the distention blocks did not differ between conditions with compared to without picture viewing, $F(1, 36.47) = 1.28$, $p = .27$.

Model 2. There was no interaction between positive or negative pictures and Pain, so these interaction terms were removed from the model, $F(1, 57.87) = 0$, $p = .99$ and $F(1, 74.39) = .18$, $p = .676$, respectively. There was no effect of viewing negative or positive pictures on startle amplitudes

compared to neutral pictures, *F*(1, 83.34) = 2.90, *p* = .09 and *F*(1, 67.78) = .58, *p* = .45, respectively. Also the main effect of gender was not significant, *F*(1, 53.72) = 1.07, *p* = .31.

A marginally significant startle potentiation was observed for the distention relative to the safe blocks (effect of Pain: *F*(1, 64.43) = 3.38, *p* = .07). The latter effect was superseded by a significant Gender x Pain interaction, *F*(1, 56.94) = 13.82, *p* < .001. Women showed startle potentiation during distention blocks relative to non-distention blocks, *F*(1, 26.71) = 14.31, *p* = .001, while startle amplitudes did not differ between distention and non-distention blocks for men, *F*(1, 30.74) = .20, *p* =.66.

Model 3. Startle amplitudes during actual inflation did not differ from those during the non-distention blocks, *F*(1, 48.25) = .61, *p* = .44. However, we found a general startle potentiation for deflation (when the balloon remained deflated in distention blocks) relative to the non-distention blocks, *F*(1, 54.17) = 19.46, *p* < .001. Females had higher startle amplitudes compared to males, *F*(1, 57.40) = 4.21, *p* = .045. Also the Gender x Inflation interaction and the Gender x Deflation interaction were significant, *F*(1, 48.25) = 14.3, *p* < .001 and *F*(1, 54.17) = 7.65, *p* = .008, respectively. Women showed startle potentiation during actual inflation, *F*(1, 25.03) = 10.49, *p* = .003, and even more during deflation, *F*(1, 29.77) = 28.04, *p* < .001, relative to the non-distention blocks. In male participants, startles were not potentiated during deflation, and showed a significant trend, opposite to females, towards decreased startle amplitudes during inflation relative to the non-distention blocks, *F*(1, 22.54) = 4.28, *p* = .05 (see Fig. 5).

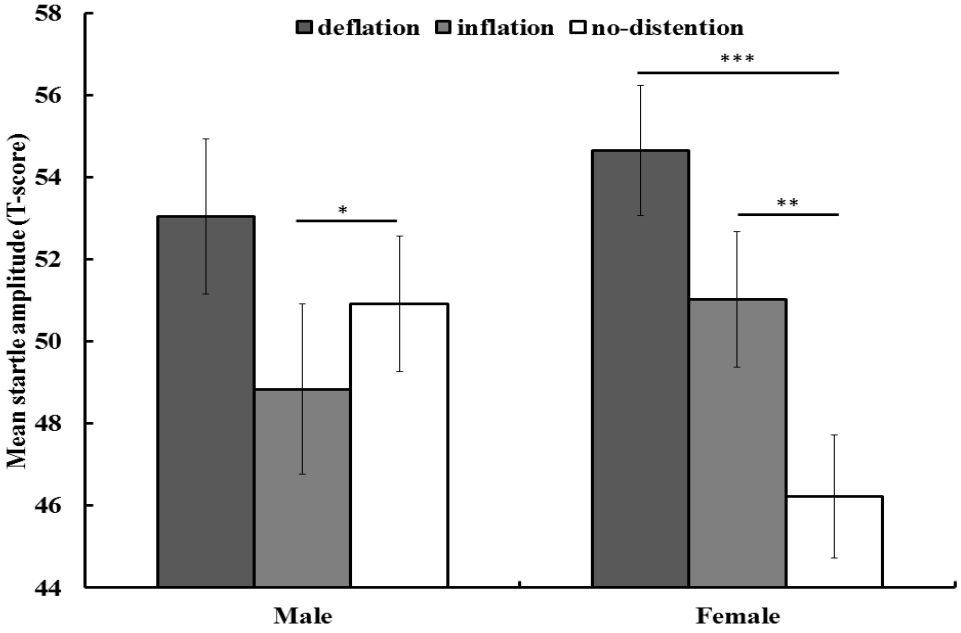

Figure 5. Startle amplitudes (mean T-scores + SE) during deflation and inflation (distention blocks) and during safe (non-distention) blocks for women and men. *** p < .001, ** p < .01, * p = .05.

4.3 Skin Conductance Levels

Table 6 displays SCLs for each block. A repeated measures ANOVA indicated a main effect of block, $F(1, 22) = 3.07$, $p = .009$, $\varepsilon = .70$, $\eta_p^2 = .12$. Following-up the latter effect indicated that skin conductance was higher during blocks with, as compared to blocks without distentions, $F(1, 22) = 14.77$, $p < .001$. However, Tukey-Kramer post-hoc pairwise comparisons indicated that this was not true for each possible pair comparing a distention with a non-distention block (see Table 6). Furthermore, Tukey-Kramer tests yielded no evidence for picture content effects.

No other effects were observed for SCL.

4.4 Evaluative judgments

Self-reported *fear* revealed a main effect of gender with women reporting higher fear than men, $F(1, 22) = 8.57$, $p = .008$, $\varepsilon = .60$, $\eta_p^2 = .28$, and a main effect of block, $F(6, 132) = 19.97$, $p < .001$, $\varepsilon = .60$, $\eta_p^2 = .48$. Figure 6 displays the marginally significant Gender x Block interaction, $F(6, 132) = 2.44$, $p = .06$, $\varepsilon = .70$, $\eta_p^2 = .09$, and suggests that with increasing levels of aversive stimulation (negative pictures and/or painful esophageal stimulation), women generally responded with a higher increase in fear compared to men.

For both pleasantness and arousal, only the main effects of block were significant, $F(6, 132) = 27.28$, $p < .001$, $\varepsilon = .57$, $\eta_p^2 = .55$ and $F(6, 132) = 9.54$, $p < .001$, $\varepsilon = .57$, $\eta_p^2 = .65$, respectively. Generally, Tukey-Kramer post-hoc comparisons (see Table 6) indicated that pleasantness was reduced for negative compared to positive and neutral picture viewing, and also reduced for blocks with compared to blocks without distentions. Furthermore, arousal ratings were influenced by picture content in the non-distention blocks (negative > neutral, positive), but not in the distention blocks.

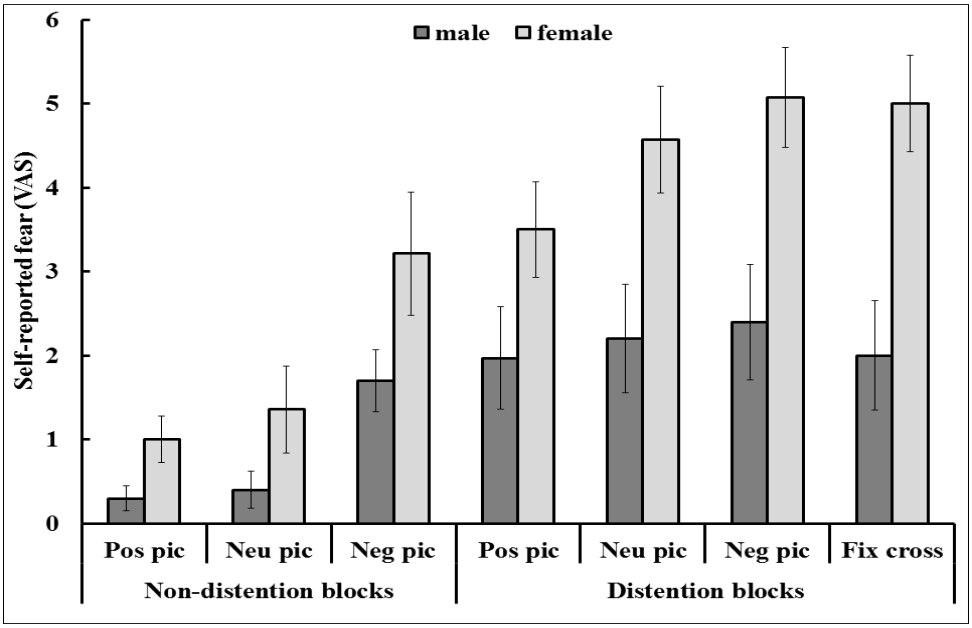

Figure 6. Self-reported fear (mean ± SE, on a 0-10 scale) of women and men for the seven blocks in the experiment.

5. Discussion

The major aim of the current study was to investigate the affective modulation of eye blink startle elicited by aversive visceral stimulation. The limited number of studies using interoceptive stimuli to induce an unpleasant affective state found a potentiation of the startle blink only during *anticipation* of the aversive interoceptive stimulus (Hubbard et al., 2011; Lang et al., 2011; Melzig et al., 2008; Naliboff et al., 2009; Pappens et al., 2013; Twiss et al., 2009). However, any such potentiation of startle was absent when startles were elicited simultaneously with interoceptive stimulation (Ceunen, Vlaeyen, & Van Diest, 2013; Pappens et al., 2010; 2012). Since most of these studies used respiratory or cold pain stimulation, it is uncertain whether these startle response findings apply to all interoceptive stimuli, or whether they are specific to these types of stimulation. To test the extent to which these findings can be generalized, we set up an experiment in which the pain stimulus was purely visceral, without stimulation of overlying somatic tissues. To this end, we applied painful inflations of a small balloon in the esophagus.

The main finding of the present study is that women, but not men, showed a fear-potentiated startle potentiation during periods of time with intermittent painful distentions. Apart from a potentiated startle, also subjective fear and pain responses to the visceral pain stimulation were stronger in women compared to men, despite pain thresholds being individually determined prior to the protocol.

As distentions could not be presented for longer than 5 seconds due to the peristalsis of the esophagus pulling the inflated balloon, blocks labeled as 'distention blocks' contained a fair amount of time during which no distentions were actually present. This warranted further exploratory analyses wherein startle responses within these blocks were grouped into those occurring in the interval between two distentions (deflated), and those occurring simultaneous with distentions (inflated). It was found that in women, startles were potentiated during both deflated and inflated conditions relative to safe blocks, although this potentiation was strongest when balloons were deflated. Men showed no startle potentiation during deflation, and a tendency towards startle *inhibition* during inflation.

These new findings add to the complex picture of startle responding during aversive interoceptive stimulation. Our findings in men are consistent with previous findings of our group on startle responding during respiratory stimuli and cold pain stimulation, during which no potentiation or even inhibition of startle was found (Ceunen et al., 2013; Pappens et al., 2010; 2012). However, the finding of an observed startle potentiation during inflation in women, was unexpected. Previously, we have argued that either attentional mechanisms or threat imminence may play a role in startle response seen during interoceptive aversive stimulation. The threat imminence explanation holds that

startle potentiation to aversive stimuli occurs only as long as no behavioral action is required – once a stimulus is so imminent that action is required or initiated, startle disappears or becomes inhibited (Lang et al., 1997; Low et al., 2008; Richter, Hamm, Pané-Farré, Gerlach, Gloster, Wittchen, Lang, Alpers, Helbig-Lang, Deckert, et al., 2012). However, our finding that men reported a smaller increase in fear to the painful distentions compared to women, is not consistent with such explanation, because higher threat imminence is not compatible with lower fear.

The alternative explanation relates to attentional mechanisms, and holds that the presence of pain or dyspnea draws attention inwards and as a consequence reduces responsivity to extraneous stimuli such as the auditory startle probe (Alius, Pané-Farré, Löw, & Hamm, 2015; Ceunen et al., 2013; Deuter et al., 2012; Pappens et al., 2010). If we would apply this explanation to our findings, then this would imply that esophageal pain does not reduce attention to extraneous stimuli in women, whereas it would in men. An argument in favor of women but not men being able to process both the startle probe and the visceral stimulus, is that women generally outperform men in multitasking (Jing, Jing, Huajian, Chuangang, & Yan, 2012; Mäntylä, 2013; Ren, Zhou, & Fu, 2009) with the exception of one study (Buser & Peter, 2012). Men on the other hand outperform women on monitoring accuracy (Mäntylä, 2013), and early research on visceral perception also suggests men are generally more accurate perceivers of interoceptive sensations (Pennebaker & Roberts, 1992). For our study, this then implies men are solely focused on monitoring their internal state, and this prevents them from being responsive to the startle probe. Of course, this speculative mechanism needs be to be further investigated, e.g., in experiments that manipulate the direction of attention (external/internal), or measure event-related potentials to the startle probes and the balloon inflations.

Although the observed effects of gender during interoceptive aversive stimulation need replication before major conclusions can be drawn, the present findings are consistent with reports in the literature on gender differences in the processing of visceral signals (Kilpatrick et al., 2010). For example, female IBS patients have been found to show greater activation than male patients in the left amygdala complex and the ventromedial prefrontal cortex during rectal balloon inflation, suggesting a greater recruitment of the fear network in response to visceral stimulation. In contrast, men responded with greater activations in the dorsolateral prefrontal cortex, insula, and PAG to this visceral stimulus, suggesting that men are more capable than women to recruit cognitive and anti-nociceptive pathways when confronted with a visceral pain stimulus (Naliboff et al., 2003). Women, but not men, showed increased activation of affective and motivational brain structures in a functional imaging study also using esophageal pain (Kano et al., 2013). Consistent with these findings, the present study found that women tended to report more intense pain than men in the distention blocks, despite the stimulation at pain threshold being individually determined prior to and kept

constant during the experimental protocol. Furthermore, although our sample may have been too small to statistically detect such interaction, the pattern of self-reported pain intensities across time (1ste, 3rd, 5th 7th distention block) suggested that women tended to sensitize to the repeated esophageal balloon distentions, whereas men tended to habituate.

Taken together, the observed gender differences in responding to painful visceral stimulation in our and other studies may be relevant to understand the higher incidence of irritable bowel syndrome (IBS) in women (Lovell & Ford, 2012), and the higher visceral hypersensitivity of female compared to male IBS patients (Mayer, Naliboff, Lee, Munakata, & Chang, 1999).

A secondary aim of the present study was to investigate whether startle modulation by affect picture viewing would be different during times without pain stimulation compared to times with intermittent painful distentions. Despite effects of picture content on self-reported pleasantness, arousal and fear, the present study did not find modulation of the startle response by affective picture viewing. Also SCL was unaffected by the affective content of the pictures. The absence of any significant difference between the positive, neutral and negative picture series on either startle or skin conductance, while leaving the subjective experience of the pictures intact, may be due to the invasive nature of the experimental context. That is, although the context may have overshadowed the effect of the pictures on *psychophysiological* responses, blocks with negative pictures were still *subjectively* perceived as eliciting more fear, unpleasantness and arousal, as the pictures were expected to do. To test whether the invasive context is responsible for this dissociation between psychophysiological responding and subjective experience, future experiments could have the participants return on a different day, and measure their response to the pictures in a setting where no invasive stimulation will take place. In that case psychophysiological responding to the picture content is expected to return.

Other than the experimental context, it is possible that the failure to elicit physiological responses to the pictures is due to the picture selection itself. Our picture set did *not* include pictures with content that elicit the strongest startle inhibition (erotica) and the strongest startle potentiation (mutilation), nor the strongest sympathetic arousal, i.e. increase in skin conductance (Bradley et al., 2001).

The picture selection may also in part be responsible for the finding that the subjective experience of positive and neutral picture series did not differ from one another on self-reported arousal and pleasantness, especially since pictures with the strongest appetitive and aversive content were not included in our selection. Examples of 'positive' picture content in our study were babies, children, athletic achievement, etc. This may explain why participants failed to distinguish between

neutral and positive pictures on all self-report measures, regardless of whether these occurred during distention blocks or distention-free blocks.

The third and last aim of the present study was to explore whether the startle blink magnitude during times of intermittent esophageal stimulation would be influenced by a concurrent picture-viewing task. Therefore, the present study also included a block with distentions in which no pictures were presented. We found no startle potentiation or inhibition in this block relative to the distention blocks with concurrent picture viewing. This is consistent with the self-report, which overall did not differ for distention blocks with pictures compared to the block without.

A first limitation of the present study is the slightly higher ratio of women compared to men. A second limitation is that we do not have information on esophageal compliance changes in response to the balloon distention, which means that we do not know whether there were individual differences in contraction force and degree of relaxation in response to stimulation. Any individual or gender related differences at this level could be also responsible for gender related differences in subjective experience and psychophysiological response measures.

In conclusion, the main finding of the present study is that in women, startle is potentiated by esophageal stimulation. We found that regardless of valence of the pictorial stimuli, participants reported higher fear, unpleasantness and arousal for the blocks with distentions, as compared to blocks without distentions. In women this was reflected in overall increased startle magnitude in the distention blocks, which is in accordance with the idea that startle is potentiated during arousing, negative affective states (Vrana et al., 1988). For both men and women, the distention blocks were more arousing than the ones without distention, which was evident from both increased electro-dermal activity and increased self-reported arousal as compared to blocks without distention.

As research on eye blink startle in response to different types of interoceptive stimulation (and esophageal and other gastrointestinal sensations in particular) has just started recently, no conclusions can yet be drawn as to the underlying mechanism responsible for the difference between startle in response to esophageal stimulation versus that to respiratory stimulation (Ceunen et al., 2013; Pappens et al., 2010; 2013). Nevertheless, exploration, description, and replications of the typical startle response occurring during different types of interoceptive stimulation may help elucidate the differential responding upon interoceptive versus exteroceptive threat. The current results may contribute to a better understanding of (1) startle responding to different types of aversive interoceptive stimulation, and of (2) gender differences in visceral pain processing. So far, both these directions of research have remained relatively unexplored, and we are confident they will receive more attention in future research.

CHAPTER 7

Visceral fear learning as a mechanism of gastro-intestinal specific anxiety

Ceunen, Zaman, Sarafanova, Vlaeyen, Van Oudenhove, & Van Diest (in preparation).

Abstract:

This study aimed to establish a new paradigm of homoreflexive interoceptive conditioning – a form of Pavlovian conditioning with a CS and US in the same sensory mode. For this, an esophageal balloon distention was used at two different intensities: one at a perceptible but non-painful intensity (CS), and one at a painful intensity (US). The paired group (N = 26) was administered 16 acquisition trials with paired CS-US presentations, that is. The unpaired group (n = 26) had 16 acquisition trials with unpaired CS-US presentations. Both groups received 16 CS-only extinction trials. Self-reported US-expectancy, skin conductance response (SCR) and startle were measured. In both groups, US expectancies increased a few seconds prior to administration of the US. The paired group also showed heightened SCRs during the CS. For men, startle during late extinction was higher at times that during acquisition were shortly prior to onset of the US.

1. Introduction

Visceral pain is one of the primary causes for seeking medical attention (Cervero & Laird, 1999; Christianson et al., 2009) and the most common form of pain resulting from disease (Cervero & Laird, 1999). Visceral pain arises from the autonomously innervated organs, i.e. the viscera, and is neurologically and psychophysiologically distinct from pain that arises from somatically innervated tissues, i.e. somatic pain (Cervero & Laird, 1999). Visceral pain or discomfort can occur following homeostatic disruption, yet a number of visceral symptoms occurs without the presence of any detectable physiological abnormalities, as is the case in functional somatic[6] syndromes (FSS). Despite that FSS are characterized by the absence of medically identifiable pathologies, there is often a history of prior damage and/or inflammation (Cervero & Laird, 1999).

Visceral pains or other discomfort seen in FSS are mostly chronic or recurrent, and can have a large negative impact on the subjective quality of life comparable to similar discomfort with a detectable cause (Henningsen, Zipfel, & Herzog, 2007). Since no physiological abnormalities are detectable, the etiology of FSS is poorly understood and makes it challenging to develop clinical treatment strategies able to alleviate discomfort caused by them. One mechanism that has been suggested to play a seminal role in the development and maintenance of FSS is interoceptive fear conditioning (Acheson et al., 2012; Acheson et al., 2007; Bouton et al., 2001; Craske et al., 2011; De Peuter et al., 2011; Mayer, 2000; Meulders et al., 2011; Pappens et al., 2013; Zaman et al., 2015). Interoceptive conditioning (IC), is a form of classical conditioning in which a relatively neutral stimulus becomes a conditioned stimulus (CS) predicting the Unconditioned Stimulus (US), and in which either the CS, the US, or both are interoceptive (Razran, 1961).

In a large portion of published IC studies, only the US is inherently interoceptive, but the CS is not. Examples of the US used in such studies are CO_2 inhalation (Fannes et al., 2008; Forsyth & Eifert, 1998; Meulders et al., 2010), breathing with respiratory resistance (Pappens et al., 2013), rectal stimulation (Benson et al., 2014; Gramsch et al., 2014; Kattoor et al., 2013), and esophageal stimulation (Yágüez et al., 2005) amongst others. In all of these examples, IC was established with either a visual or olfactory CS. These paradigms can be relevant to understanding panic disorder with agoraphobia, multiple chemical sensitivity, and something as simple as learned food preferences.

In a recent study, non-painful stimulation of the distal esophagus served as CS, while a cutaneous painful electric shocks served as US (Zaman et al., submitted). While some may consider this as a form of IC wherein the CS is interoceptive and the US is not (intero-exteroceptive conditioning), we argue it is an IC study with two interoceptive, but functionally distinct stimuli

[6] The 'somatic' in FSS refers to the body as a whole (identical to its use in the words 'somatoform' and 'psychosomatic'). This is not to be confused with the narrower definition of 'somatic' that is used when distinguishing visceral and somatic tissues as based on efferent innervation.

(heteroreflexive IC). This study demonstrated that learned fear to a benign esophageal sensation can generalize to other intensities of esophageal stimulation (Zaman et al., submitted).

Most relevant to FSS, however, may be when the CS is in the same sensory mode as the US and is also a natural precursor of a US. This is referred to as homoreflexive conditioning (Bouton et al., 2001; Razran, 1961) (sometimes also called homotopic conditioning (Dworkin, 2007)). Homoreflexive conditioning is of particular interest to understanding FSS, because in FSS originally benign visceral sensations become associated with unpleasant visceral sensations.

The purpose of the current study was to establish a homoreflexive interoceptive conditioning paradigm applying stimulation of the esophagus as both CS and US, with CS and US only differing from one another in intensity of stimulation. Apart from establishing a new conditioning paradigm, this study was also intended to test the commonly made assumption that fear conditioning processes can occur between two visceral sensations. Although seminal in theories on FSS, panic disorder, and anxiety disorders, this assumption thus far has not been put to the test thoroughly, with less than a handful of published studies of research on homoreflexive interoceptive fear conditioning in humans (Acheson et al., 2012; Acheson et al., 2007; Pappens et al., 2013). Without exception, all these homoreflexive conditioning paradigms involved respiratory stimulation, while to date there are no publications on homoreflexive conditioning paradigms involving stimulation anywhere along the alimentary tract. The limited number of studies on this particular topic emphasizes the need for expanding the knowledge base regarding homoreflexive IC. Ultimately, such studies may contribute to the development and improvement of clinical treatment strategies for FSS and related disorders.

For the paradigm in the current study, the esophagus was chosen as site of stimulation as it has a clear distinction between somatic and autonomic innervation in respectively the upper and lower parts and has corresponding differences in cortical processing (Aziz et al., 2000b). Unlike some other forms of visceral stimulation, stimulating the lower esophagus allows for applying visceral stimulation without activating stretch receptors in overlying somatic tissues. To provide translational value for (functional) visceral pain syndromes, the US in the current study was intentionally at an intensity of stimulation that was subjectively painful. A subjectively detectable non-painful stimulation at the same distal site of the esophagus served as a CS. Physiological responses (startle eye blink potentiation and galvanic skin responses) as well as on-line US-expectancies were recorded throughout in order to shed more light on the effects of the conditioning paradigm applied. We expected fear learning to the CS to occur when the CS precedes the painful US (paired group), but not when the CS and US are separated by a relatively long time interval (unpaired group). Because sex differences in visceral processing have been described in the literature (Benson et al., 2014; Kano et

al., 2013; Labus et al., 2013; Pennebaker & Roberts, 1992), we considered it relevant to include gender in our analyses.

2. Methods

2.1. Participants

Fifty-two healthy participants (26 women) were recruited via advertisements on social media. Individuals who expressed an interest in participating, received an informed consent prior to deciding whether or not to participate. The informed consent was in line with the declaration of Helsinki (World Medical Association, 2008): it outlined the experimental procedure including stimuli to be undergone, guaranteed anonymity, stated that participation was voluntary (with a reimbursement of 50 Euros), and mentioned that participation could be halted at any moment if the participant so desired, without loss of the promised reimbursement.

Individuals who desired to participate were required to indicate whether or not they had a history or presence of: (a) psychiatric conditions such as anxiety disorders, somatization disorders, depression, addiction related disorders or other; (b) abdominal or thoracic surgery (excepted appendectomy and cholecystomy); (c) neurological, endocrine, or digestive disorders, and/or (d) other medical disorders. Moreover they also had to indicate whether at the time of the experiment they (e) were pregnant, (f) had pain symptoms, (g) used medication that influences the function of the digestive tract and/or the nervous system, (h) had a recent accident of which they weren't fully recovered yet, and/or (i) had a serious hearing impairment. If interested individuals responded affirmatively to any or several of these, they were deemed unfit for participation and where kindly thanked for their interest in participating. Individuals who agreed to participate and who met the requirements, were requested to abstain from smoking, and abstain from drinking alcoholic and caffeinated drinks starting the evening prior to participation; they were advised to participate with their stomachs neither too full (to prevent vomiting), nor completely empty (to prevent faintness).

Participants were assigned to one of two groups: a paired and an unpaired group. In the paired group, the Conditioned Stimulus (CS) was presented and followed almost immediately by an Unconditioned Stimulus (US); i.e. in this group the CS and US were 'paired' in all of the learning ('acquisition') trials in which there was both a CS and a US. In the unpaired group, there was an interval of 26 seconds between the CS and the US onset; thus in this group the CS and the US were 'unpaired' in all trials in which both a CS and US was administered. The labeling 'paired' and 'unpaired' as well as the design of this experiment are inspired by earlier published research (Pappens et al., 2014; Pappens et al., 2013) in which the paired and unpaired groups were designed to differ with respect to whether or not the CS announced the imminent occurrence or absence of the US. Thus, whereas the CS

announces the imminence of the painful US in the paired group, it announces an imminent 'safe' and pain-free period in the unpaired group. In contrast to the studies described by Pappens et al. (Pappens et al., 2014; Pappens et al., 2013), in the current study the interval between CS and US was kept constant. Participants of both genders were evenly distributed over both groups (13 women and 13 men in each group), and ages were approximately the same in both groups with mean age (standard deviation) of participants 22.2 y/o (1.4) in the paired group and 22.7 y/o (2.5) in the unpaired group. Approval for conducting the experiment was obtained from the Medical Ethical Committee of the University of Leuven (reference number ML8570).

2.2. Stimuli

Both the CS and US consisted of mechanical stimulation of the distal, autonomously innervated part of the esophagus. To be able to stimulate this site, a pediatric catheter (diameter of 3mm, Pennine Healthcare) was inserted via the nose until 35cm from the nostril. The internal part of the catheter had a small, deflated rubber balloon attached to it at 2.5cm from the end. In order to prevent any part of the catheter from moving during the experiment, the external part of the catheter was attached to the face using adhesive tape. An air-filled syringe with a volume of 50ml was attached to the external part of the catheter, and served as a means to inflate and deflate the esophageal balloon. The CS and the US lasted 5 and 2 s, respectively.

The intensity of stimulation was individually determined for both CS and US using a variation of the ascending methods of limits. For this threshold determination, the volume of the balloon increased with 1 ml relative to each previous inflation. Between each 1 ml inflation, the balloon was deflated. For each inflation subjects were required to indicate whether they felt something, and to rate what they felt on a scale from 0 to 10, with zero being no sensation at all, 1indicating possibly a sensation (not being entirely certain), 2 indicating a sensation definitely being present but not yet painful, 8 being a clearly painful but still tolerable sensation, and 10 being the maximally tolerable intensity of pain. Subjects were warned that intensity 10 would never be used, and that it was always possible to reduce the volume if the subjective intensity was too high. During threshold determination, up to and including intensity 3, the balloon was inflated for 5 seconds, equaling to the duration of the CS to be used in the experiment. Beyond this point on the scale, the balloon was inflated for two seconds only, equaling to the duration of the US to be used in the experiment. The entire threshold determination procedure was repeated a second time to make sure the thresholds were accurate. In case the second threshold determination yielded different results than the first, the thresholds obtained during the second determination were used. The intensity corresponding to number 2 on the scale (i.e., perceivable, but non-painful) was used as a CS throughout the experiment, while the intensity corresponding to number 8 (painful, but bearable) was

used as US. These individually determined thresholds did not yield significant differences between genders for the mean volumes for either CS (14.87 ml; 14.85 ml for women, 14.88 ml for men) or US (30.06 ml; 28.77 ml for women, 31.35 ml for men).

2.3. Measures

All signals described below (US expectancy ratings, startle EMG, GSR) were recorded using Affect 4.0 software (Spruyt et al., 2010) and transmitted via A 16-Bit PCI-6221 data acquisition card (National Instruments, Austin, Texas) to a computer, and treated offline with Psychophysiological Analysis software (PSPHA) (De Clercq et al., 2006).

2.3.1. Subjective expectancy of US onset

Throughout the study, participants had their dominant hand on a custom-built dial (Pappens, Smets, et al., 2012; Vansteenwegen, Iberico, Vervliet, Marescau, & Hermans, 2008), and were required to continuously rate the extent to which they expected the US in the following seconds. The scale of the dial visually ranged from 0 to 100. A score in the middle (50) meant the participant totally did not know whether or not to expect the US. The more participants turned the dial below 50 and towards zero, the more certain they were that the US would not come. The more they turned the dial from 50 upwards to 100, the more certain they were to expect the US. The position of the dial in the scale was digitally registered at 10Hz and transmitted via a data acquisition card to a computer throughout the entire experiment.

The recorded digital values give an indication of the subjective estimation of each participant on how likely they felt they were to receive the US in the following seconds. As such, it can be used to assess whether participants learned to make correct predictions of US onset.

2.3.2. Psychophysiological measures

Eye blink startle

Eye blink startle is a somatic response which, when elevated, is thought to reflect defensive motor preparation to aversive or fear inducing stimuli. It was elicited and measured as based on the guidelines of Blumenthal et al. (Blumenthal et al., 2005). More specifically, a 50ms burst of white noise with a volume of 102dB was used as an acoustic startle probe. Eye blink startle was measured using electromyographic (EMG) recordings of the orbicularis oculi muscle using two electrodes placed next to one another on the left lower eyelid, and with a ground electrode on the center of the forehead. All three of these reusable electrodes were filled with high conductivity water-soluble Microlyte™ electrolyte gel. Sites where electrodes were attached were first cleaned with alcohol to remove the horny layer of the skin and any possible make-up, thereby reducing inter-electrode skin impedance and improving signal strength.

The raw EMG signal was amplified by a LabLinc v75-04 Coulbourn Isolated Bioamplifier with Bandpass filter; the recording bandwidth was between 13Hz and 1 kHz. This signal was transmitted to a LabLinc v76-24 Coulbourn 4 Channel Integrator which rectified and smoothed the signal online with a time constant of 20ms. The EMG signal was digitized at 1 kHz starting 500ms prior to onset of the acoustic probe, until 1000ms after probe onset.

Skin Conductance Response (SCR)

The SCR is a measure of sympathetic and emotional arousal (Dawson et al., 2007). After cleaning the hypothenar side of the non-dominant hand with alcohol, two standard Ag/AgCl electrodes (diameter 1cm) filled with water-soluble KY*gel were attached here, spaced approximately 2.5cm apart. The galvanic skin response measured via these electrodes was transmitted to the LabLinc v71-23 Coulbourn Isolated Skin Conductance Coupler, which maintained a constant voltage of 0.5V over the electrodes; the analog signal was digitized at 10Hz.

2.4. Procedure

Upon arrival participants were welcomed by both a male and a female experimenter, the former wearing casual clothing and the latter wearing a white lab coat. After going through the informed consent again (which subjects had read prior to participating) and after having the participant accustomed to the lab, the catheter with the deflated esophageal balloon was inserted by the medically trained female experimenter. When participants indicated they had accustomed to the presence of the catheter, individual thresholds for CS and US were determined according to the procedure described in section 2.2. Then, electrodes for measuring startle and SCR were attached (see section 2.3.2), while subjects were verbally informed what these electrodes were used for. This information included mention about the occurrence of acoustic startle probes throughout the experiment. Following electrode attachment, the intended use of the dial was explained to participants, and after they indicated they had no more questions, earphones were mounted on their head. Finally, the male experimenter left to an adjacent room to monitor the signals, and returned to the lab only after termination of the experiment.

Throughout the entire experiment the female experimenter remained in the lab with the participant in order to be able to administer the CS and US when required. Inflation and deflation of the esophageal balloon occurred outside the field of vision of the participant for both CS and US, by means of a manually operated air filled syringe. The experimenter administering the CS and US was cued to do so via a monitor, which was also placed outside the field of vision of the participant. On this monitor, a countdown occurred 5 seconds prior to inflation while indicating whether a CS or US

had to be administered, and a second countdown occurred starting from onset of inflation, showing the remaining time till deflation.

In first instance, participants had to habituate to the acoustic startle probe that they would get to hear at different times throughout the experiment. This is necessary as the startle magnitude in response to the startle probe tends to be exaggerated upon initial presentation, and becomes more stable after repeated stimulation (Blumenthal et al., 2005). To establish habituation, twelve probes were administered with a fixed interval of ten seconds immediately prior to the onset of the actual experiment.

After habituation, the participant had to start using the US expectancy dial as described in section 2.3.1 and had to continue doing so until the end of the experiment. The dial was fixed in place within arm's length in front of the participant. The experiment consisted of three phases, although the participant did not have explicit knowledge about this: it included (a) a pre-exposure phase, (b) an acquisition phase, and (c) an extinction phase. Every single trial in all three of these phases lasted 48 seconds, with acoustic probes being administered at the 19^{th} and 43^{rd} second after trial onset. The CS (i.e., light esophageal stimulus) was administered from the 15^{th} up to the 20^{th} second after trial onset. The CS was preceded and followed by an inter-stimulus interval (ISI). ISI here refers to any period in a trial longer than 2 seconds without esophageal stimulation. Only in the acquisition phase of the experiment, an additional US (i.e., painful esophageal stimulus) was administered, lasting 2 seconds (fig. 7). In the acquisition phase, for the paired group the acoustic probe delivered at 19 seconds was 3 seconds prior to US onset, and the probe at 43 seconds was in a 'safe' period, while for the unpaired group the opposite was true. In other words, for each acquisition trial, a first startle probe was administered during the CS, so that in the paired group, this probe was three seconds before the onset of the US. A second probe was delivered during the end of the $ISI_{post\text{-}CS}$, so that for the unpaired group the second probe was three seconds before onset of the US.

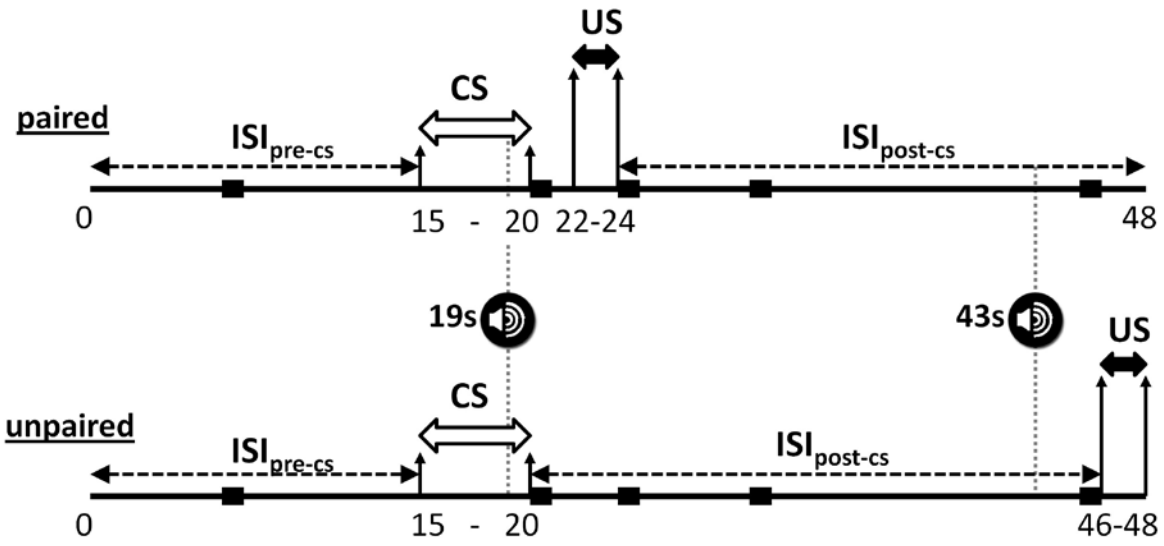

Figure 7. Trial timelines

Schematic illustration of the timelines of reinforced trials during the acquisition phase for the paired and unpaired groups, respectively the upper and lower timeline. The Conditioned Stimulus (CS) was delivered from 15 to 20 seconds after trial onset, and preceded and followed by an inter-stimulus interval (ISI), respectively labeled $ISI_{pre\text{-}cs}$ and $ISI_{post\text{-}cs}$. An Unconditioned Stimulus (US) was delivered from 22 to 24 seconds after trial onset for the paired group, and from 46 to 48 seconds for the unpaired group. The sound symbols represent acoustic startle probes, which were invariably administered at 19s and 43s after trial onset. The black squares on the timelines are the points in time which were included in the analysis of the subjective US-expectancy (see section 3).

The pre-exposure phase consisted of four trials, with one CS administered during each trial from the 15th up to the 20th second after trial onset, totaling four CS administrations during the entire pre-exposure phase. (This phase is labeled pre-exposure as it occurred prior to the phase in which the participants were routinely 'exposed' to the US.)

The second phase was the acquisition phase, the only time during which the US was administered (other than during individual threshold determination performed prior to the experiment). The acquisition phase consisted of 16 conditioning trials. For each four trials, there were three reinforced trials and one non-reinforced trial. In a reinforced trial the CS was followed by a US while in a non-reinforced trial, the US was absent. In the reinforced trials in the paired group, the US followed almost immediately after the CS, from the 22nd to the 24th second, while in the unpaired group, the US occurred only after the inter-stimulus interval ($ISI_{post\text{-}cs}$), starting at the 46th second, i.e. 26 seconds after CS onset. In the entire acquisition phase, there were a total of 12 reinforced, and 4 non-reinforced trials. This proportion of non-reinforced trials relative to reinforced trials actually

strengthens the effect of conditioning, especially when the non-reinforced trials occur at unpredictable moments (Phelps, Delgado, Nearing, & LeDoux, 2004). To make the occurrence of non-reinforced trials unpredictable for participants, they were pseudo-randomized; for all participants non-reinforced trials occurred during the 3rd, 8th, 11th, and 15th trial of the acquisition phase.

The final phase of the experiment consisted of an extinction phase, which paralleled the acquisition phase in number of trials and CS administrations (16 in total), but during which the US was no longer administered. As its name indicates, it served to extinguish any learned or 'acquired' predictive connection between CS and US. After the extinction phase, the experiment was concluded and stimulus and measurement electrodes were removed.

3. Data reduction and analysis

As US-expectancy was measured continuously, data were reduced by selecting 5 time points of interest for each trial: the 7th second (middle of $ISI_{pre\text{-}CS}$[7]), 20th second (end of CS), 24th second (end of US for the paired group), 33rd second (middle of $ISI_{post\text{-}CS}$[8]) and the 45th second (right before US onset for the unpaired group). The US-expectancies at these selected time points were used for analyses.

Skin conductance responses (SCRs) were calculated by subtracting the mean skin conductance level (SCL) during baseline (2s before the CS onset) from the maximum value in the window 0-7 s following CS onset. After SCRs were averaged across trials, SCR-data were $LOG_{10}(1 + SCR)$-transformed before being analyzed.

Eye blink startle (EMG) responses were calculated by taking the difference between the peak value in the 21 - 175 ms time window and the mean value from the 0 -20 ms time window following probe onset. All startle responses were T-transformed within persons to correct for inter-individual variability that was unrelated to the experimental conditions of interest.

One block was created for the pre-exposure phase comprising all four trials. To enable visualizing the evolution of acquisition or extinction learning, two blocks (Early / Late) comprising 8 trials each were created for each phase.

Hypotheses were tested with planned comparisons in repeated measure ANOVAs with the following factors: Group (paired, unpaired), Gender (male, female), and Block (pre-exposure, early acquisition, late acquisition, early extinction, and late extinction). Only Group and Gender were between subject variables. For US-expectancy, also a factor 'Time' was included (7th, 20th, 24th, 33rd, 45th second) with the 7th second as reference. For startle EMG, also a factor Stimulus (CS, $ISI_{post\text{-}CS}$) was included in the rm ANOVA design. Main and interaction effects of the omnibus repeated measure

[7] $ISI_{pre\text{-}cs}$ = Inter Stimulus Interval from trial onset till onset of the CS.

[8] $ISI_{post\text{-}cs}$ = Inter Stimulus Interval from CS offset till US onset in the unpaired group.

ANOVAs will not be reported, except for significant effects of Gender (as we did not formulate no apriori hypotheses regarding the effects of Gender).

To test the main hypothesis that fear learning to the CS would occur and extinguish again in the paired relative to the unpaired group, we defined for each measure a set of planned contrasts testing for group (paired/unpaired) differences in each phase separately or in changes from one phase to another. For US-expectancy, we tested for each phase whether both groups differed in their change in US-expectancy from baseline (7th second) to the end of the CS (20th second). We expected that compared to the unpaired group, the paired group would have a stronger increase in US-expectancy from baseline (7th second) to the end of the CS (20th second) in the acquisition, but not both other phases.

For SCR, contrasts were created to assess group differences in SCR during each phase (pre-exposure, acquisition and extinction); we expected group differences (paired > unpaired) to occur only during acquisition.

For startle EMG, planned contrasts were created to assess whether CS amplitudes increased relative to ISI amplitudes from pre-exposure to acquisition in the paired group compared to the unpaired group. Furthermore, we tested whether the CS-ISI difference in the paired group disappeared from late acquisition to extinction compared to the unpaired group.

To allow for a further exploration of the data, other, we also run a few *un*planned contrasts applying a Bonferonni correction for multiple testing. Alpha was set at .05. Greenhouse-Geisser corrections were applied where appropriate. Uncorrected degrees of freedom and corrected *p*'s are reported together with η_p^2. All statistical analyses were performed using SPSS 20.

4. Results

4.1. US-expectancy

4.1.1 Pre-exposure

During pre-exposure, groups did not differ on the change in US-expectancy from the 7th second to the 20th second $F(1,48) = .301$, $p = .586$ or from the 7th second to the 45th second $F(1,48) = .047$, $p = .829$. Post-hoc tests indicated that US-expectancy rating were not increased significantly at the 20th relative to the 7th second in either group (paired: $F(1,48) = .048$, $p = .827$; unpaired: $F(1,48) = .992$, $p = .324$) .

4.1.2 Acquisition

During acquisition, US-expectancy ratings increased from the 7th second to the 20th second in the paired group compared to unpaired group $F(1,48) = 3.316$, $p = .037$*, $\eta_p^2 = .065$. A post-hoc test indicated that US-expectancy ratings at the 45th second relative to the 7th second were significantly

higher in the unpaired compared to the paired group $F(1,48) = 10.453$, $p = .001$*, $\eta_p^2 = .179$ (*see Fig. 8*).

4.1.3 Extinction

During extinction, groups no longer differed in changes in US-expectancy ratings from the 7th second to the 20th or the 45th second ($F(1,48) = .34$, $p = .563$; $F(1,48) = .382$, $p = .540$), although there was still a tendency for US-expectancy ratings to be increased at the 20th second compared to the 7th second in the paired group $F(1,48) = 5.649$, $p = .022$, $\eta_p^2 = .105$ whereas in the unpaired group this was not the case $F(1,48) = 2.41$, $p = .127$) (*see Fig. 8*).

4.1.4 Gender effects

No effects of gender were observed (all p's = n.s.).

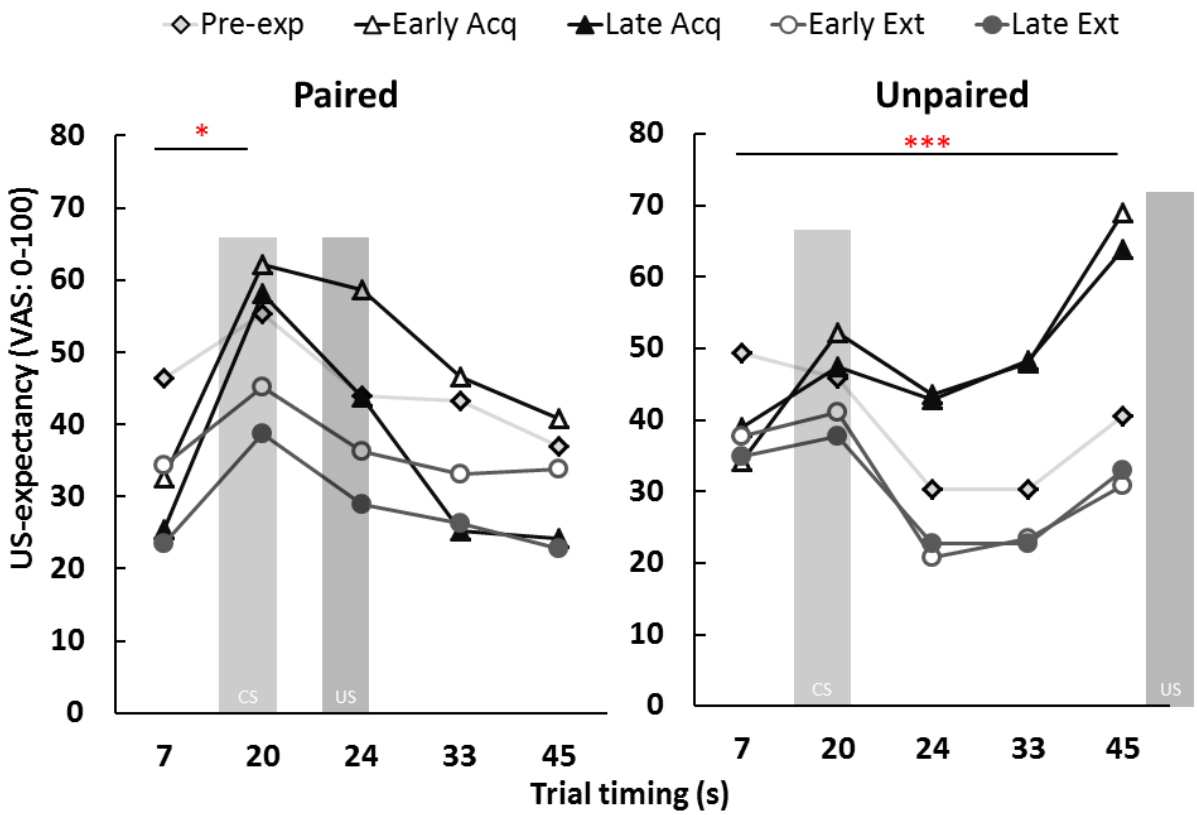

Figure 8. US-expectancy

Mean US-expectancy ratings at the 7th, 20th, 24th, 33rd and 45th second for the paired and unpaired group during pre-exposure (Pre-exp), early acquisition (Early Acq), late acquisition (Late Acq), early extinction (Early Ext) and late extinction (Late Ext). The light grey bars represent the presentation of the CS, the darker grey bars represent the presentation of the US.

4.2. SCRs

4.2.1. Pre-exposure

During pre-exposure SCRs to the CS did not differ between groups $F(1, 48) = .251$, $p = .619$.

4.2.2. Acquisition

During acquisition, SCRs to the CS were significantly higher in the paired group compared to the unpaired group $F(1,48) = 5.125$, $p = .014$*, $\eta_p^2 = .096$. Post-hoc tests indicated that SCRs to the CS habituated more in the unpaired group from pre-exposure to early acquisition compared to the paired group $F(1,48) = 4.095$, $p = .049$, $\eta_p^2 = .079$ (*see Fig. 9*).

4.2.3. Extinction

During extinction, no group differences were observed on SCRs to the CS $F(1,48) = 2.598$, $p = .114$. A post-hoc tests showed that from late acquisition to late extinction SCRs decreased in the paired group compared to the unpaired group $F(1,48) = 4.174$, $p = .043$, $\eta_p^2 = .08$ (*see Fig. 9*).

4.2.4. Gender effects

There was a significant Block*Gender interaction $F(4,192) = 3.059$, $p = .05$, $\eta_p^2 = .06$, that originated from more habituation to the CS from pre-exposure to early acquisition in women compared to men $F(1,48) = 4.137$, $p = .048$, $\eta_p^2 = .079$). However, women and men did not differ in SCRs to the CS in any block (all p's = n.s).

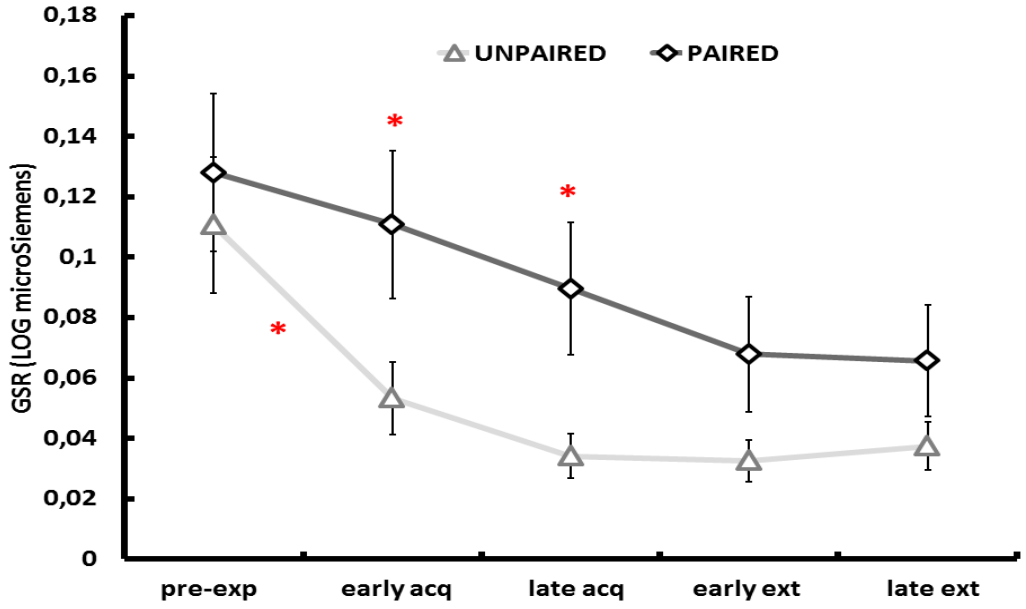

Figure 9. SCR

*Mean log transformed Galvanic Skin Responses (GSR) of the paired and unpaired group during pre-exposure, early and late acquisition, early and late extinction. The error bars represent the standard error. * p ≤ .05*

4.3. Startle eye blink EMG

4.3.1. Pre-exposure

During pre-exposure no group differences were observed in startle amplitudes to the CS relative to startle amplitudes during the $ISI_{post\text{-}CS}$ $F(1, 48) = 1.767$, $p = .190$.

4.3.2. Acquisition

From pre-exposure to acquisition there was only a marginally significant trend towards increased startle amplitudes during the CS relative to the $ISI_{post\text{-}CS}$ for the paired group compared to the unpaired group $F(1,48) = 1.975$, $p = .088$, $\eta_p^2 = .04$.

4.3.3. Extinction

From late acquisition to extinction (early + late extinction **combined**) there were no group differences in the change of CS amplitudes relative to $ISI_{post\text{-}CS}$ amplitudes $F(1,48) = .933$, $p = .339$. However, during late extinction, there was a strong group effect on the CS- $ISI_{post\text{-}CS}$ difference $F(1,48) = 9.208$, $p = .004$, $\eta_p^2 = .161$: startle amplitudes to the CS were higher compared to $ISI_{post\text{-}CS}$ amplitudes in the paired group, whereas in the unpaired group the opposite was observed (*see Figure 10A & 10B*).

4.3.4. Linear effects

Based on Figure 9 and the significant Group*Block*Stimulus interaction $F(4,192) = 2.695$, $p = .032$, $\eta_p^2 = .053$, we also explored linear trends across experimental blocks for each level of Group and Stimulus. For the unpaired group, we observed a significant increase in $ISI_{post\text{-}CS}$ startle amplitudes across blocks $F(1,48) = 18.754$, $p < .0001$, $\eta_p^2 = .281$, which was not present for the CS startle amplitudes $F(1,48) = 0.92$, $p = .763$. The linear effects in the CS or ISI startle amplitudes of the paired group were not significant after correction for multiple testing (CS: $F(1,48) = 4.202$, $p = .046$, $\eta_p^2 = .081$; $ISI_{post\text{-}CS}$: $F(1,48) = 1.389$, $p = .244$.

4.3.5 Gender effects

Gender modulated the effect of group on the pattern of change of CS- $ISI_{post\text{-}CS}$ startle amplitudes differences across blocks (Block*Stimulus*Group*Sex interaction; $F(4,192) = 2.424$, $p = .050$, $\eta_p^2 = .048$). To further explore the role of gender we tested for each gender separately the effect of group on changes in CS- $ISI_{post\text{-}CS}$ differences from pre-exposure to acquisition and extinction. For men, from pre-exposure to acquisition there was no effect of group on CS- $ISI_{post\text{-}CS}$ differences $F(1,48) = 0.42$, $p = .839$. That is, from pre-exposure to acquisition no increase in CS amplitudes relative to $ISI_{post\text{-}CS}$ amplitudes was observed in the paired group compared to the unpaired group. For women, from pre-exposure to acquisition there was an effect of group on CS- $ISI_{post\text{-}CS}$ differences $F(1,48) = 4.803$, $p = .033$, $\eta_p^2 = .091$. Startle amplitudes during the CS increased relative to ISI amplitudes from pre-exposure to

acquisition in the paired group compared to the unpaired group of women (*see Fig. 10 E & F*). However, this effect in women did not survive the correction for multiple testing. Furthermore, for men, CS amplitudes during late extinction were higher compared to ISI amplitudes in the paired group, whereas in the unpaired group ISI amplitudes were higher compared to CS amplitudes $F(1,48) = 14.926$, $p < .001$, $\eta_p^2 = .237$ (*see Fig. 10 C & D*).

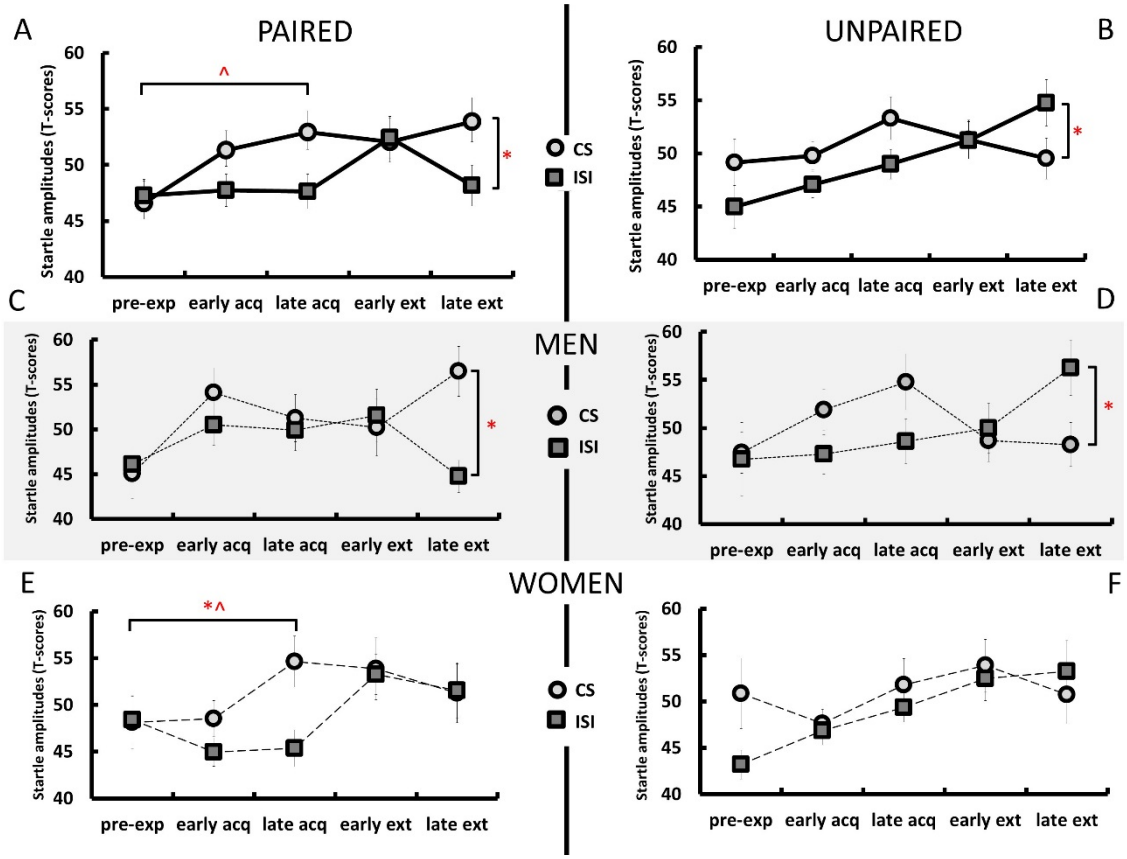

Figure 10. Startle

*Mean startle amplitudes (T-scores) for the (A) paired group and the (B) unpaired group, irrespective of gender. Mean startle amplitudes for men in the (C) paired group and (D) unpaired group and for women in the (E) paired and (F) unpaired group. CS = conditioned stimulus; ISI = Inter-stimulus interval (after CS); acq = acquisition; ext = extinction. The error bars represent the standard error. * p ≤ .05.*

5. Discussion

The purpose of the current study was to investigate fear learning to a visceral CS using a novel, homoreflexive interoceptive conditioning paradigm. This is especially relevant to the understanding of FSS and ultimately may contribute to the clinical treatment thereof, given that homoreflexive IC has been hypothesized to play a prominent role in the etiology of FSS. Prior to this study, the only homoreflexive interoceptive conditioning paradigms described in the literature involved respiratory stimulation (Acheson et al., 2012; Acheson et al., 2007; Pappens et al., 2013). Although conditioning

paradigms with painful stimulation at different sites along the alimentary tract have also been conducted and described in the literature previously, none of these were homoreflexive (Benson et al., 2014; Gramsch et al., 2014; Kattoor et al., 2013; Yágüez et al., 2005). I.e., although the US in these studies was a painful visceral stimulus, the CS was not the same type of visceral stimulus below pain-threshold. Therefore, the design of the current study can provide unique insights not previously explored, with both the US and the CS in this study as visceral stimuli administered at the same anatomical site, namely at the distal esophagus.

We hypothesized that fear learning to the visceral CS would be evident from increases in expectancy of the US when the CS would immediately precede the painful US (paired group), compared to when the CS would never followed directly by US (unpaired group), and also that it would be evident in increased skin conductance (a measure of sympathetic and emotional arousal) and a fear potentiated startle. Moreover, since there are numerous indications that genders differ in the processing of visceral stimuli (Benson et al., 2014; Kano et al., 2013; Labus et al., 2013; Pennebaker & Roberts, 1992), the current study was optimized for further investigating any possible gender effects by recruiting an equal number of male and female participants.

5.1. Pre-exposure and Acquisition

During acquisition in both the paired and the unpaired group, US-expectancy increased right before onset of the US. This increased expectancy of the US was accompanied by a relatively higher SCR during the CS in acquisition in the paired group as compared to the unpaired group. In contrast, in the unpaired group, there was a marked drop in SCR in response to the CS from pre-exposure to early acquisition, which was not seen in the paired group.

In the paired group, by the latter half of the acquisition phase, startle amplitudes in response to the CS increased relative to the amplitudes in response to the CS during the pre-exposure phase. In this same group, amplitudes in response to the $ISI_{post\text{-}CS}$ did not change from pre-exposure to late acquisition. The unpaired group showed the opposite pattern, with increases in startle amplitude during the $ISI_{post\text{-}CS}$, but not during the CS. Somewhat puzzling is that, when we included gender, the effects observed in startle at this phase of conditioning seemed driven by the female participants. The learning curve from pre-exposure to late acquisition in the paired group, indicates that only women tend to develop a differential startle response to CS and $ISI_{post\text{-}CS}$, whereas men's startle responses do not show a differential pattern during this phase.

Another finding of note is that during pre-exposure and throughout acquisition, in the unpaired group, startle amplitudes during the CS were somewhat higher than those during the $ISI_{post\text{-}CS}$. This is somehow unexpected because in the unpaired group there was never an objective contingency between CS and US; in fact, if there was a contingency it was a negative contingency, with

the CS signaling a US-free period. That startle during CS was nevertheless somewhat higher than during $ISI_{post\text{-}CS}$ is likely due to the homoreflexive nature of the paradigm in which the CS intrinsically refers to US. Though SCRs in the unpaired group indicate lower emotional and/or sympathetic arousal, startle suggests that even in this group the CS is never considered completely safe prior to extinction. The subjective US-expectancy in this group supports this interpretation, as it is relatively high throughout most of the trials in acquisition as compared to the paired group. This implies that benign visceral sensations resembling painful or otherwise aversive visceral sensations are more readily associated with one another than that heterotopic sensory input is associated with a visceral US.

Taken together, the findings indicate that participants in both groups learned to anticipate the onset of the US largely correct, as reflected in their subjective expectancy ratings, their SCRs and to some extent, startle. This in turn supports earlier hypotheses on the etiology of gastro-intestinal anxiety which attribute an important role to associative learning processes by which initially relatively 'neutral' bodily sensations start provoking fear (Acheson et al., 2012; Acheson et al., 2007; Bouton et al., 2001; Craske et al., 2011; De Peuter et al., 2011; Mayer, 2000; Meulders et al., 2011; Pappens et al., 2013). Moreover, the absence of safety learning in the unpaired group indicates that stimuli which are inherently similar to the US are likely associated with it, even when in fact they signal a safe period. This is in line with other research that found that a light esophageal stimulation as CS becomes generalized to visceral stimulation at other intensities (Zaman et al., submitted).

5.2. Extinction

During extinction, the US expectancy ratings of paired and unpaired groups were more or less equal. Though SCRs showed an overall strong habituation as time progressed, SCRs during early extinction were still relatively higher in the paired group as compared to the unpaired group. Thus it seems that the extinction phase did not inhibit the association that had earlier been formed between CS and US. The lack of extinction learning has also been previously observed by Pappens, Smets et al (2012).

Startle results also indicate that the association between CS and US was not effectively inhibited. For the paired group, the startle response during late extinction was the same as during late acquisition. That is: a stronger startle during CS than during $ISI_{post\text{-}CS}$. For the unpaired group however, the startle pattern during late extinction was opposite to the pattern this group exhibited during late acquisition. Specifically, during late extinction in the unpaired group the startle in response to the $ISI_{post\text{-}CS}$ had become larger than the startle in response to the CS. Including gender in the analyses revealed these effects on startle during extinction were only evident in men.

That the association between CS and US did not effectively extinguish may be attributed to a number of factors. There is (a) the perceptual likeness of the CS to the US, with the light sensation of

the CS naturally occurring during the build-up of the strong sensation of the US, even if just for a fraction of a second. This likeness may make it difficult to inhibit the association between the CS and the US, despite that in the current study the distinction between CS and US was made perceptually salient by the short time interval between CS and US. The presence of the time interval brings us to another possible explanation for the findings (b). The current paradigm involves trace conditioning. In trace conditioning the CS ends before the US begins, in contrast to forward and delayed conditioning, where the CS ends only after onset of the US. Extinction of learned associations is known to be generally slower in trace conditioning (Ewald et al., 2014). A final possibility that may explain why associations between CS and US failed to extinguish (c), is due to the partial reinforcement scheme where one out of four trials in acquisition were not reinforced. Partial reinforcement not only strengthens the effect of conditioning (Phelps et al., 2004); it also makes individuals learn that the absence of a US in one trial does not signal the absence of the US in subsequent trials. Under such relatively unpredictable conditions, the CS may be expected to precede the US at any time, even if the US has not been presented for several trials. Thus there is never really certainty whether the US will still at one time follow the CS or not. In summary: perceptual likeness, trace conditioning, and partial reinforcement may all contribute to the failure to extinguish the previously learned CS-US association in the paired group.

5.3. Linear effects on startle

As for the extinction phase in the unpaired group, it was observed that the startle response to $ISI_{post\text{-}CS}$ became stronger during extinction, although neither the subjective ratings nor the SCRs indicate an increased anticipation of the unpleasant US during $ISI_{post\text{-}CS}$. When including all phases from start to end of experiment, it can be seen that for the unpaired group there was a linear increase in startle responsiveness to the $ISI_{post\text{-}CS}$. This linear increase resulted in startle to the $ISI_{post\text{-}CS}$ being highest in late extinction relative to all other phases of conditioning. A trend towards a similar linear increase was observed in the paired group for startle in response to the CS.

The observed linear increase in startle across the experiment for both paired and unpaired groups is very unusual, as startle responsiveness typically decreases over time, even after the initial habituation phase (Acheson et al., 2007; Bouton et al., 2001). We argue that the presence of a linear increase in responsiveness is most likely in part the result of a shift in attention allocation. Previous studies on startle and interoception indicate that when attention is allocated internally to aversive interoceptive sensations, startle responses are relatively smaller than when attention is not entirely devoted to just these sensations (Alius et al., 2015; Ceunen, Vlaeyen, et al., 2013; Pappens, Van den Bergh, Vansteenwegen, & Van Diest, 2011). In line with these findings, we argue that in our experiment initially the painful esophageal distentions naturally draw attention inward due to their

relative novelty while reducing processing of the startle probe, while over time it gradually becomes possible to process and respond to all stimuli –including the startle probe, which translates into the observed linear increases in startle amplitudes.

5.4. Gender differences

As mentioned in sections 5.1 and 5.2, there were marked gender differences in startle responsivity during respectively acquisition and extinction. During acquisition, only women from the paired group tended to show an increased startle during the CS. In contrast, in late extinction it were only the men who displayed increased startle to the probe that in the acquisition phase had preceded the US. These marked gender effects seen in startle add another dimension to the findings discussed earlier, and may appear difficult to interpret given that no parallel gender differences were observed in SCRs and objective US-expectancy.

The only gender difference in SCR was that women's SCR initially habituated more than that of men. As galvanic skin responses are generally more responsive and elevated in men (Dawson et al., 2007), the observed gender difference in SCR in our study is not all that unusual, thus cannot be taken as an indication that the CS had no effect on women. The gender effects observed in startle are meaningful and indicative there are genuine, notable gender differences in processing of visceral signals. Although no gender differences were observed in aware, objective US-expectancy, this does not exclude the possibility of gender differences in US-expectancy on a deeper, autonomous, sub-aware level of processing which is reflected in startle.

The present findings on gender differences in startle responding are based on a 4-way interaction, and may therefore be underpowered and reflect a spurious finding. Still, gender differences in the processing of visceral signals have been observed many times prior to this study (Camilleri & Choi, 1997; Kano et al., 2013; Kilpatrick et al., 2010; Labus et al., 2013; Naliboff et al., 2003; Pennebaker & Roberts, 1992; Stark et al., 2006). One imaging study in particular that may shed light on the gender differences we found in startle, is that of Benson and colleagues (Benson et al., 2014). They applied a conditioning paradigm not unlike ours, and also used a visceral US. The major differences between their study and ours is that their US was a rectal and not esophageal distention, that their paradigm was exteroceptive (the CS was a visual stimulus), and that instead of an unpaired control group, they had a differential conditioning paradigm, meaning that certain visual stimuli (CS+) were paired with the US, while other visual stimuli (CS-) were never paired with the US. Benson and colleagues found that women show greater activation of the insula than men during late acquisition; this neural response is interpreted as greater anticipation of the CS+ in women than in men. Other research links greater activation of the insula in women to a preponderance of the affective and motivation components of visceral pain in women. That in our study only the women, but not men in

the paired group had a bigger startle response during the CS than during $ISI_{post\text{-}CS}$ by the time they got to the late acquisition phase, indicates that these women had a strong affective component when they anticipated the US that men did not have. Earlier research also indicates that startle is increased during unpleasant affective anticipation of aversive interoceptive stimulation (Lang et al., 2011; Melzig et al., 2008).

Gender effects were also seen during late extinction, with only men responding with greater startle to the CS in the paired group, and greater startle to the $ISI_{post\text{-}CS}$ in the unpaired group. This may be due to gender specific activity in the posterior cingulate cortex (PCC) during extinction, with women generally showing reduced PCC activity (Benson et al., 2014). In rats, a lesion of the PCC impairs the learning of associations during late acquisition (Bussey, Muir, Everitt, & Robbins, 1996). In visceral pain conditioning in humans, the PCC is thought to be involved in encoding the emotional component of cues predicting pain, and cues predicting safe, pain-free periods (Gramsch et al., 2014). Based on this, it can be hypothesized that reduced PCC activity during extinction may reduce the association between the pain predictive cue and the US, and between the safe cue and the absence of the US. As women generally have reduced PCC activity during extinction relative to men, our startle data during extinction indicate that women did no longer specifically associate the pain predictive cue with the US, nor the safe cue with the absence thereof. Instead, women's startle was generally elevated during both CS and $ISI_{post\text{-}CS}$. In contrast, the men in our study had a relative higher startle amplitude during the cue that previously predicted the US, and a lower startle to the 'safe' cue in late extinction. These findings strongly suggests that after acquisition of a CS-US link, women, but not men tend to have a generalized affective anticipation of the US, even though on a subjectively aware level, men and women do not show differences in reported expectancy. This generalized affective anticipation in turn may be associated to the female preponderance of FGIDs - disorders in which homoreflexive interoceptive conditioning is thought to play a crucial role in both onset and maintenance.

Alternatively, gender differences in startle may be due to gender differences in the ability to divide attention. Building on the argument in section 5.3, there is a possibility that in the acquisition phase, men's attention is oriented toward their internal state, making them less responsive to the acoustic startle probe, while women's attention is better divided between internal state and other sensory input. Thus, women, but not men show increase in startle amplitude during acquisition in response to the probe that most often immediately precedes the US. It can further be argued that as the threat imminence becomes less in extinction, men become more responsive to acoustic probes. However, attention alone does not account for why women and men do not respond alike during extinction with regards to startle, thus urging us to still resort at least in part to the arguments in the two previous paragraphs.

5.5. Limitations

There are some limitations to our study. As mentioned in section 5.5, the present findings on gender differences in startle responding are based on a 4-way interaction. This means the possibility exists that these findings are underpowered and reflect a spurious finding, even though the literature suggests there are gender differences in processing of visceral stimuli.

Moreover, we need to remain careful in drawing conclusions in how the current conditioning paradigm translates to conditioning of genuine *fear* of interoceptive sensations. Although we have data on self-reported fear for the CS (available upon request), the conditioning paradigm did not have any effect on subjective fear experienced during the CS. Generally in lab situations, even those involving invasive aversive stimuli such as ours, subjects usually do not report much fear. This may be because of social desirability, a sense that serious risks in this environment are most likely minimal, and in our case probably also the self-selection mechanism at work, which most likely precluded individuals with high anxiety sensitivity to volunteer for participation in this study. It would certainly be unethical to induce fear learning in the lab that approximates genuine pathological interoceptive fear, without having a sufficient guarantee one will be able to extinguish the established fear.

5.7. Conclusions

Despite this limitation, we can draw some relevant conclusions from our findings. We have established that homoreflexive interoceptive conditioning with an esophageal stimulus, can effectively induce associative learning. We have also established that despite the presence of an actual negative contingency between CS and US in the unpaired group, the CS is not considered entirely safe. Both findings provide support for theories which hypothesize a role of homoreflexive conditioning in the arisal of FSS. Additionally, that participants never indicated to be absolutely sure no US would come, as well as the relative difficulty of extinguishing the startle response, showcases that conditioned associations between a benign visceral and a painful visceral sensation are hard to extinguish. Finally, the gender differences we found in startle are suggestive of gender differences in processing of visceral stimuli, and can be connected to the preponderance of women in FSS. However, our findings on gender differences need replication with greater samples of both genders.

Although we do not advise future studies to induce genuine fear prior to establishing an effective method for extinction of learned associations, ecological validity can be further increased by employing our homoreflexive paradigm using other visceral sensations, namely in the stomach or rectum to more closely resemble the sensations typical in FGIDs. Of further interest in relation to FGIDS is whether visceral conditioning affects visceral perception, i.e. whether it affects perception ~, discomfort ~, and/or intolerance thresholds.

Part III

General Discussion

CHAPTER 8

General Discussion and Conclusions

Interoceptive fear is the apprehension of bodily sensations. It is present in somatoform, anxiety, and mood disorders, yet it is unknown whether interoceptive fear can reliably be assessed using startle. Aversive visual, auditory, and olfactory stimuli have been shown to induce potentiation (an increase in magnitude) of startle via a mechanism referred to as affective modulation (Bradley & Lang, 2000; Ehrlichman et al., 1995; Jansen & Frijda, 1994; Vrana et al., 1988). Fear too induces potentiation of startle through a mechanism called fear potentiation of startle (M. Davis, 2006). Regardless of whether a stimulus is truly fear inducing, or merely unpleasant but not feared: if it is accompanied by sufficient arousal, it will potentiate the startle reflex (Bradley et al., 2001).

The major aim of this doctoral project was to examine whether the human eye blink startle paradigm can be used to measure defensive response mobilization during background *interoceptive* stimulation. The eye blink startle paradigm has been validated using a range of emotional stimuli and is widely used as a psychophysiological measure to assess motivational direction (pleasantness-unpleasantness) and defensive response mobilization (which is reflected in fear potentiated startle). Thus far, the usefulness of this paradigm using interoceptive stimuli has received scant attention. Previous studies as well as our own studies indicate that when using aversive interoceptive stimuli, there is quite some variability in startle responding that does not uniformly fit the usual pattern of affective modulation or fear potentiation (Ceunen, Vlaeyen, et al., 2013; Pappens, De Peuter, et al., 2012; Pappens et al., 2010).

To better understand interoceptive fear, in this project not only a variety of paradigms were used, but also a variety of measures were included. Apart from startle, measures included self-report to assess the subjective experience of participants during interoceptive stimulation. Additionally, skin conductance was measured in half of our studies, as a proxy of emotional and sympathetic arousal. The results for each of these measures are discussed under separate headings in this chapter, after a review of each of the studies and their aims below.

1 Overview of studies and their aims

Study 1: Effect of seated trunk posture

The aim was to determine whether postures associated with dyspneic sensations (extension of the spine) and with gastro-intestinal sensations (flexion of the spine) have any effect on startle, and whether they are subjectively different from one another. First, earlier findings found startle potentiation during gastrointestinal stimulation (Schächinger et al., 2009), but not during dyspneic stimulation (Ceunen, Vlaeyen, et al., 2013; Pappens, De Peuter, et al., 2012; Pappens et al., 2010). As the former is associated with flexion (Sikirov, 2003) and the latter with extension (Honig, 1990) of the spine, we aimed to investigate whether involvement of postural muscles could be responsible for these results. Alternatively, postures affect emotional state (Duclos et al., 1989; Flack, 2006; Riskind & Gotay, 1982), and as a consequence perhaps alter startle. Moreover, the whole body startle is a reflex involving spinal flexion (Landis & Hunt, 1939; Yeomans et al., 2002), and posture has been shown to affect this whole body reflex (Brown et al., 1991). Although two studies have investigated whether posture also affects the associated eye blink reflex (Price, Dieckman, et al., 2012; Joe Wielgosz et al., 2012), neither has manipulated spinal curvature in an otherwise affect free context.

Thus, study 1 was set-up to investigate whether posture could be responsible for the difference in startle observed during gastric stimulation as opposed to that during respiratory stimulation. Participants in this study had to assume three different postures for several minutes each; one posture with the spine flexed, one with the spine upright, and one with the spine extended. Eye-blink startle was measured during all three postures, and self-report was administered after each posture.

Study 2: Cold pain and respiratory stimulation

While affective modulation of startle has been observed in anticipation of interoceptive stimulation (e.g., Hubbard et al., 2011; Lang et al., 2011; Melzig et al., 2008; B. Naliboff et al., 2009; Pappens et al., 2013; Twiss et al., 2009), startle measured during actual interoceptive stimulation had only been scarcely reported prior to this study. Startle during breathing with a resistance, during inhalation of CO_2-enriched air, and during thermic pain stimulation have all been studied previously. The few studies on this suggest startle is paradoxically not elevated during interoceptive stimulation, but rather reduced. We were interested to know how startle would behave at different time points during prolonged stimulation. An earlier study from our group suggested that startle progressively reduces in response to CO_2. There were also indications that the affective component of pain during immersion of the hand in cold water fluctuates depending on time since immersion, and thus it could be hypothesized that perhaps so does the startle. However, the effect of cold pain on startle over time

had not been tested previously. For breathing against a resistance (loaded breathing), no data were available at the time on how startle behaves when loaded breathing occurs over a prolonged interval. Our study was the first to administer all three of these interoceptive stimuli within subjects.

Study 3: Visceral pain

Further attempting to elucidate the startle response topography to interoceptive stimulation, the distal esophagus was mechanically stimulated at individually determined pain threshold. As previous studies on gastric stimulation did find a potentiation of startle, this gave rise to the question whether startle at other sites along the alimentary tract would behave alike, or whether they would show the reduced startle as found in response to aversive respiratory stimuli. The esophagus was chosen as site of stimulation as it has clearly distinct somatic (proximal part) and visceral (distal part) innervation, and allows for visceral stimulation without stimulating overlying somatic tissue.

Of additional interest was whether the phenomenon of affective pain modulation can be extrapolated to visceral pain. Another additional aim was to find out whether affective state is still reflected in startle in the context of visceral pain, as visceral pain itself was expected to induce a negative emotional state.

The major and minor aims study 3 were addressed by including six time intervals –i.e., blocks – were affect was induced with a series of affective pictures of one valence per block. Half of these picture blocks were with, and half without the esophageal stimulation. A seventh picture free block with esophageal stimulation was added in order to observe the effect of visceral pain in the absence of concurrent visual mood stimuli. Startle and skin conductance were measured in each block, and self-report at the end of each block assessed subjective fear, valence, arousal and pain intensity.

Study 4: Visceral fear learning

While earlier research as well as our own research suggests that startle potentiation does not occur for a range of aversive interoceptive stimuli, other research suggests that during interoceptive conditioning (IC), startle potentiation does occur (Pappens, Smets, et al., 2012; Pappens et al., 2013). In these IC studies startle potentiation occurred in response to an interoceptive stimulus when this stimulus immediately preceded a more aversive interoceptive sensation.

Visceral fear learning through IC has been proposed as a mechanism involved in the etiology and maintenance of somatoform disorders (Acheson et al., 2012; Acheson et al., 2007; Bouton et al., 2001; Craske et al., 2011; De Peuter et al., 2011; Mayer, 2000; Meulders et al., 2011; Pappens et al., 2013; Zaman et al., 2015). When CS and US are both in the same sensory mode, this is referred to as homoreflexive IC. However, the few studies on homoreflexive IC prior to this study have only used respiratory stimulation.

The purpose of study 4 was to establish a homoreflexive interoceptive conditioning paradigm using esophageal stimulation at two different intensities; one at detection threshold (CS) and another at a subjectively painful intensity (US). In a between subject design, a control group was included and was administered the same stimuli, but with a different temporal contingency. The primary information on participants' subjective expectancy of the US was obtained by letting participants continuously rate the extent to which they expected the US to occur. Skin conductance was measured as an indication of arousal. In line with the doctoral project's main aim, startle was included as a measure so we could further map the startle response topography to interoceptive stimuli. In this study it concerned the startle response to conditioned interoceptive stimuli, prior to learning of associations, during learning, and during extinction. Because study 3 found gender differences in startle in response to esophageal stimulation, study 4 was optimized to investigate potential gender differences, and had an equal ratio of men to women.

2. Startle modulation

Study 1: Effect of seated trunk posture

Startle amplitudes were higher during the extended posture than during the flexed or the upright posture. State negative affect induced by a posture was associated with higher startle amplitudes, regardless of posture. On a subjective level, the extended posture was also different from the two other postures, being more unpleasant and arousing, and inducing less feeling of control, more discomfort, and more perceived difficulty. The study also showed that positive affect associated with a posture does not modulate startle. Interestingly, the difficulty of the posture led to a decrease in startle when all other factors were held constant. This study indicates that postures can modulate startle, because of their associated negative affectivity (leading to an increased startle). In contrast, the difficulty of a posture –arguably an interoceptive component of the posture - can decrease startle.

It is premature to try and relate these findings to prior research, as the effect of posture on startle has hardly been examined. The few earlier studies on startle and posture (Price, Dieckman, et al., 2012; Wielgosz, Repshas, Greischar, & Davidson, 2012) differ too much from ours to allow for a comparison or integration of the findings. The startle modulation found in our study is most likely due to the extended posture being unnatural and requiring a lot of continued muscular effort of relatively untrained muscles. Unless participants in studies using interoceptive stimuli are required to sit in an unnatural position, we argue that it is unlikely posture is responsible for the variation in startle responses seen in studies with interoceptive stimuli.

Study 2: Cold pain and respiratory stimulation

Overall, potentiation of startle was absent, with startle responsivity showing a tendency to the opposite direction for most stimuli, i.e. decreased magnitudes relative to no stimulation. Despite this general startle pattern, all of these stimuli were experienced as aversive, reasonably fearful, and arousing. When looking at the startle pattern for each individual stimulus, we can observe the following. From study 2 it became clear that for respiratory stimuli there is a linear decrease in startle magnitude relative to the duration of the stimulus since its onset. Although cold pain on average also led to a decrease in startle responsivity, startles administered at different times during the cold pain stimulus did not all behave alike, and displayed some time related fluctuations. That is: with three startle probes delivered at three different times during prolonged cold pain, the startle amplitude was not equal at all different time points since onset of stimulation. Previous research has found that the pain experience during the cold pressor test also varies during the course of stimulation (Lovallo, 1975) and can explain why startle amplitudes at different moments since the onset of cold pain are not alike. To test this explanation, future studies should add an online (i.e., continuous) measure of pain.

Study 3: Visceral pain

The results of the third study show that affective visual stimuli do not modulate the startle blink when presented in the context of esophageal stimulation. However, unconditioned painful stimulation of the distal esophagus does modulate startle. Men and women differ in startle responsivity, with women having an increase in startle magnitude in blocks with esophageal stimulation relative to blocks without such stimulation. This increase in startle during blocks with stimulation was evident both when stimulation was on and when it was off. In contrast, startle magnitudes of men were smaller during actual stimulation, relative to stimulation free blocks. This gender difference in startle responding could not be observed in study 2, because that study was comprised of only women. The gender differences in startle during study 3 were not only limited to startle; fear was generally higher in women than in men. Thus, in study 3 there was an apparent fear potentiated startle in women. However, no other self-report measures, nor skin conductance levels in study 3 showed any gender differences.

Study 4: Visceral fear learning

The fear conditioning paradigm was successfully established. This was clear from accurate self-reported US expectancy, and heightened skin conductance during the CS for the paired group. As in study 3, in study 4 gender differences also emerged. During late extinction, men, but not women had significantly higher startles at times that previously indicated US onset was imminent. I.e., in late extinction men in the paired group had a higher startle during the CS. This implies that an interoceptive stimulus (CS) can co-occur with an increased startle amplitude when it is predictive of an aversive

stimulus (US). Statistically less significant effects also support this view. I.e., irrespective of gender, in the acquisition phase the paired group also showed a trend towards increased startle magnitudes during the CS. When testing for gender effects, this effect was seen in women but not men. Although statistically insignificant, this further indicates that startle during interoceptive stimulation (CS) can be heightened when predictive of something aversive (US). That effects during acquisition were insignificant, does not necessarily indicate there are no effects; instead the statistical power may have been insufficient.

3. Possible modulatory influences on startle

Taken together, the above results allow us to make some direct and indirect conclusions regarding startle response patterns during interoceptive stimulation. First, even though the interoceptive stimulations we used were unpleasant, arousing and fearful, this often did not translate into startle potentiation. Second, when there was startle potentiation, it was usually only observed in one gender. Third, in the homoreflexive conditioning paradigm (study 4) in the paired group during acquisition, there was an increase in startle during the CS (an originally non-aversive) for the paired group for women (but not significant after correction), and for men during extinction (significant).

From all these direct observations, it becomes clear that startle measured during different types of interoceptive stimulation is not necessarily modulated by fear or unpleasantness alone. Although the potentiation seen in women in study 3 could be attributed to fear, and study 1 showed that negative affect associated with a posture is reflected in increased startle, the remainder of the results still begs for interpretation. To be able to do so, we are required to take into consideration any other known modulatory influences on startle. At least two such modulatory influences could be relevant here and help explaining the results. One of these modulatory influences is arousal, while the other is attention (Bradley & Lang, 2007).

In paradigms where startle was administered during anticipation of a stimulus affecting the mood, or during mental imagery of an emotional scenario, startle reflects arousal rather than valence. During anticipation of an emotional stimulus, irrespective of whether it is a pleasant, a feared or an unpleasant stimulus, startle will be potentiated (Nitschke et al., 2002; Sabatinelli, Bradley, & Lang, 2001; Skolnick & Davidson, 2002). A similar effect has also been observed during mental imagery, where startle is higher when the motivational intensity (arousal) of the visualized scenario is high (Miller, Patrick, & Levenston, 2002; Robinson & Vrana, 2000; VanOyen Witvliet & Vrana, 1995). In study 4, the arousal linked to anticipation of the US could be responsible for the marginal increase in startle during acquisition in response to the CS in the paired group. Indeed, skin conductance

measures do indicate that SCRs in response to the CS were in fact higher in the paired group during acquisition.

The reduced startle during interoceptive background stimulation as found throughout different paradigms can also be due the modulatory influence of attention. Attention can exert both excitatory as well as inhibitory influences on startle. Inhibitory influences have been observed during early processing of the startle stimulus: this is referred to as prepulse inhibition (PPI) (Braff, Geyer, & Swerdlow, 2001). Other than the PPI phenomenon, direction of attention may also modulate startle. When attention is oriented to a sensory modality congruent with the startle probe, normal affective modulation of startle is found. However, when attention is directed to a sensory modality at the exclusion of the sensory modality in which the startle probe is presented, this leads to a reduced startle responsiveness (Pappens et al., 2011). As interoceptive stimuli naturally draw attention to the state of the body, this could explain why startle was reduced, rather than increased in many instances.

However, this fails to explain why there are gender differences. If startle is reduced because it draws attention inward, why doesn't it appear to draw attention inward in women in the context of esophageal stimulation (where startle amplitudes are high)? (See study 3) One answer could be that it is because women are naturally better at dividing their attention (Jing et al., 2012; Mäntylä, 2013; Ren et al., 2009). Although this may answer why we observed gender differences, it doesn't answer why there were differences in startle in different studies. Specifically: if women show startle potentiation in the context of esophageal stimulation, then why don't women also show startle potentiation in face of respiratory stimuli and cold pain? Thus far, we do not have a satisfactory answer to this question, although the following paragraphs make an attempt.

An important difference between the second and third study is that in study 2 each stimulus (respiratory load, CO_2-enriched air, cold pressor) was administered only once for a relatively long time, while in study 3 stimulation (of the esophagus) was brief but administered repeatedly. When taking this difference into consideration from the perspective of attentional modulation of startle, this could be taken to imply that prolonged interoceptive stimulation reduces responsivity to external stimuli such as the startle probe, but that brief repeated interoceptive stimulation does not. To test any effect duration of stimulus presentation, and repeated versus single presentation may have, it suffices to manipulate these in future explorative studies. Unfortunately esophageal stimulation cannot be administered for prolonged durations due to peristaltic contractions, and inhalation of CO_2-enriched air is less likely to induce any sensation if only administered during a single inhalation. The duration of loaded breathing and immersion of the hand in cold water can more easily be manipulated, so as to match that of esophageal stimulation in a within subject design.

Alternatively, it could be that differences in processing of different interoceptive sensations are responsible for differences in startle. In chapter one it was argued that interoception is to be used as an umbrella term to refer to the phenomenological perception of the body, and that different components of interoception allow us to classify a variety of interoceptive sensations as alike on some aspects, but also as different from one another. In this light, we can attempt to identify components that unite all stimuli in study 2 (respiratory load, CO_2-enriched air, cold pressor) and which distinguish them from the esophageal stimulus in study 3.

Apart from a difference in duration, and the number of times a stimulus was presented, the esophageal balloon distention differed from the other stimuli in that it stimulated solely visceral tissue, while all other stimuli involved a combination of visceral and somatic stimulation as well as visual and auditory feedback. The respiratory stimuli themselves were not visible, but inhalation and exhalation elicited movement in valves visible to the participant. Furthermore, inhaling through a mouthpiece can make the participants' breathing more clearly audible to herself. The cold pressor was also within the field of vision and was accompanied by a continuous audible sound emitted by the thermoregulatory which also circulated the water. In contrast, the esophageal stimulus was not accompanied by any visual or auditory feedback. Once inserted it was completely out of the field of vision of the participant as the extraneous part of the catheter was attached to the face with tape; manual distention of the esophageal balloon also occurred out of the field of vision and was entirely inaudible.

In other words, the respiratory and cold pain stimuli were perceived via a multitude of sensory faculties and centrally integrated in the phenomenological percept, while the perception of the esophageal stimulus relied entirely on visceral tissue afferents. Possibly, when a multitude of sensory channels all contribute to a single percept – namely the state of the body – this leaves less cognitive resources for processing sensory input not related to this percept. In contrast, when there is only a single sensory channel that contributes to interoception, it is more likely there are still enough attentional resources available for processing of non-interoceptive sensory information such as the startle probe. To examine this, a study could be set up involving purely visceral (esophageal or rectal) stimulation on the one hand, and on the other the same visceral stimulation but in co-occurrence with somatic, visual and auditory feedback. In case of the latter, less processing resources are expected to be available, and the responsivity to the startle probe should be reduced relative to the former.

In addition to the modulatory influences of arousal and attention on startle, there are two other potential explanations for the observations we made regarding startle responses. One is the defense cascade model of Lang and colleagues (1997), and the other is the perceptual-defensive-recuparative model of Bolles and Fanselow (1980).

According to the defense cascade model, fear potentiated startle is only evident during the post-encounter phase. Once threat is so imminent that it shifts the defensive fear response from post-encounter to circastrike -a phase characterized by offensive/defensive action - there is no more startle potentiation (Richter, Hamm, Pané-Farré, Gerlach, Gloster, Wittchen, Lang, Alpers, Helbig-Lang, & Deckert, 2012). In this light then, the absence of startle could be interpreted as the interoceptive stimuli eliciting circastrike responding. Although we cannot exclude this entirely for study 2, in study 3 this is an unlikely explanation for the reduced startle found in men. It is unlikely because the fear reported by men in study 3 was lower than that of women. Moreover, men tended to habituate to pain while women tended to sensitize. Additionally, if participants would truly have reached the circastrike phase, this would reflect in a tendency to action (escape from the stimulus or at least a tendency to move), extreme fear and hyperventilation. This was not observed.

The perceptual-defensive-recuparative model of Bolles and Fanselow (1980) does not directly make reference to startle, but it does make reference to fear. In particular, based on animal research it states that fear is a defensive behavior, while pain induces recuperative behaviors. Most importantly, this model postulates that recuperative behaviors and fear responding are mutually exclusive. The 'perceptual' aspect of the model refers to the models' axiom that recuperative behaviors enhance perception of nociceptive stimulation. (This could be taken to mean that attention is oriented to nociceptive processing.) On the other hand, fear enhances perception of events in the environment to facilitate the detection of danger and the detection of options for safety.

In chapter one we argued that pain is merely a form of interoception; based on this we could extrapolate the model of Bolles and Fanselow from its original focus on pain to also apply more broadly to unpleasant interoception in general. This would mean that homeostatic disturbances as elicited by our interoceptive stimuli might elicit recuperative behaviors and increased processing of the interoceptive stimulus, while inhibiting fear responding. Pain recuparative behaviors include resting and body-care responses to promote recovery from injury. Generalized to interoception, recuperative behaviors could refer to anything that is aimed at restoring homeostasis (including not only voluntarily, but also autonomous responses, as will be clear from the examples in the next paragraph).

For each of the stimuli in our study, we can think of at least one recuperative behavior. For example, loaded breathing induces a sensation of reduced airflow. To compensate for the reduced air intake one could increase respiratory effort, while the sensation of reduced airflow could be compensated for by reducing the number of breaths. Inhalation of CO_2 enriched air leads to an increase in the partial pressure of arterial CO_2 which can be compensated for by hyperpnoea. Hyperpnoea refers to increased minute ventilation appropriate to the acidotic state and is aimed at removing excess CO_2. With immersion of an extremity in cold water as in the cold pressor test (CPT),

the most natural recuperative behavior is to get the extremity out of the cold. When that is not an option, the body may initiate autonomic responses, but we need to take into account these may not necessarily be appropriate to this particular stimulus. Selective pressure in human evolution has led the body to prevent prolonged cold from damaging the organs by means of peripheral vasoconstriction. While peripheral vasoconstriction may occur during the CPT, peripheral vasodilation of the immersed hand would perhaps be a more appropriate homeostatic response. Peripheral vasodilation may not occur until the hand is removed from the water. And finally, a recuperative behavior to deal with sensations of esophageal discomfort could be to adjust salivary production, swallowing frequency and/or contractile force during swallowing.

A conclusion loosely based on the model of Bolles and Fanselow (1980) could be that aversive interoceptive stimulation in some cases precludes normal fear responding because it induces recuperative responses that are incompatible with fear responses and defensive mobilization in general. (Their model states that recuperative behaviors and fear behaviors are mutually exclusive.) This would mean that aversive interoceptive stimulation precludes fear potentiation of startle. However, this explanation is not entirely in accordance with the original perceptual-defensive-recuparative model. The original model states that extreme levels of fear will always override pain and induce fear analgesia. Yet, when we want to be able to interpret the absence of startle as being due to increased interoceptive perception and the initiation of associated recuperative behavior, it would have to imply that interoception can override fear. If this is true, then future research efforts should be aimed at understanding which factors could amount to recuperative behavior overriding fear, and which factors amount to fear overriding recuperative behavior. Based on the original model of Bolles and Fanselow, it could be concluded that acute pain and higher levels of fear prioritize fear responding over recuperative behavior. However, this may not hold true when pain is prolonged or when experiencing non-painful homeostatic disturbances and when fear is not high. Indeed, in women prolonged cold pain and dyspnea did not lead to a fear potentiated startle, while brief periods of pain in the esophagus did lead to a relatively increase in fear in women (as compared to men) and associated increase in startle amplitude.

As of yet, it is still unclear which of the above mechanisms are responsible for the startle response patterns as observed in our studies. We will discuss in a later section how we can address this.

4. Skin Conductance

A phasic rise in skin conductance is common in response to a discrete arousing stimulus, while a tonic rise in skin conductance is common in response to a prolonged arousing stimulus (Michael E. Dawson

et al., 2007). These conclusions were originally based on studies using exteroceptive stimuli. However, previous data from our research group indicate that skin conductance responses also occur in reaction to arousing respiratory sensations (Pappens, De Peuter, et al., 2012; Pappens et al., 2010). The skin conductance measures in this doctoral project as applied in study 3 and 4 further support that increases in skin conductance also occur in response to arousing interoceptive stimuli.

In study 3, skin conductance levels were generally higher during blocks with esophageal distention. Self-reported arousal levels were also generally higher during distention blocks. In study 4, skin conductance responses in response to the CS were higher during acquisition for the paired group than for the unpaired group. This corresponded with increased US-expectancy ratings for this group during the CS. These findings indicate that skin conductance is a reliable measure of arousal, also when used in experimental paradigms with interoceptive stimulation.

5. Fear and unpleasantness ratings

A note should be made about the study of interoceptive fear. To study this concept, generally self-report measures need to be included, and ideally these measures indicate that we are indeed measuring the constructs we hope to measure, and that our manipulations are successful in inducing the emotional states we intend to study. Apart from the use of a fear scale, other measures may serve a complementary role to this purpose. Scales that inquire about valence, arousal, and dominance (perceived control), and in conditioning studies the measure of US-expectancy (Boddez et al., 2013), can all be used to measure interrelated constructs. Generally, fear is a negative emotional state (unpleasant), it is arousing, and is more likely to occur when dominance is low. Likewise, in fear conditioning studies, it may be more informative to inquire about US expectancy than it would be to inquire about fear, as participants may not necessarily label their aroused US-expectancy as fear. Based on this reasoning, Study 4 indicates that fear can be learned to a visceral sensation. Participants learned to accurately predict when the US would come. In the paired group, the interoceptive CS became a predictor of the interoceptive US. This was clear from subjective online US-expectancy ratings. Startle and skin conductance in study 4 corroborate that US-expectancy results can be interpreted as evidence of fear learning, as also startles and skin conductance increased in response to the CS in the paired group.

While fear levels in study 2 and 3 were not low, they were also never high enough to reach the maximum end of the fear scale. This can be due to a number of factors, but is definitely not merely a result of response bias. There may be a general reluctance to label one's own emotional state as fear. Although participating in research can initially create a state of arousal, participants are usually well informed on the stimuli they will be administered and can find comfort in the knowledge that the

studies have been approved by an ethical committee and are considered safe. In such an environment, it is understandable that fear does not reach extreme levels. However, this does not make our results irrelevant to the study of fear: fear is a construct that is difficult to measure. We do believe that the self-report included in our studies provides sufficient information to conclude that the stimuli used can considered close enough to eliciting interoceptive fear to an extent that is still very ethical towards participants.

6. Limitations and future research

The dominant limitation of the current project was its explorative nature. This itself is not a direct limitation, as it allows to get a better view on the startle response pattern. However, the nature of exploration is that of observation, while explanation of the observations is only of secondary importance at such an initial stage. It is only after making initial robust observations that attempts can be made to elucidate the mechanisms responsible for the observed effects. In case of this project, we were interested in mapping the startle response pattern to a variety of interoceptive stimuli, in different paradigms. As we did not have sufficient knowledge on what to expect, studies could not be optimized to understand and critically test the mechanism responsible for the observed startle patterns.

Future studies should generally be designed while keeping in mind that startle is not only affected by motivational direction (pleasant-unpleasant) and threat, but also by other factors. Presenting an aversive interoceptive stimulus, while manipulating the levels of subjective arousal could allow us to understand the impact arousal has on startle in interoceptive fear. Manipulating orientation of attention, or at least measuring orientation of attention (see figure 11) could provide further information on the role attention has in the modulation of startle in the context of aversive interoceptive stimulation. To investigate the possibility that absence of startle potentiation is due to circastrike responding, additional physiological measures such as heart rate and respiration could be included. However, the danger with including a variety of peripheral measures is that this does not necessarily ease interpretation. The most challenging to investigate is whether recuperative tendencies could under some circumstances be inhibiting fear potentiated startle, as this requires us to predict recuperative behaviors beforehand and to come up with a way to quantify these.

A major point of attention that came forward from our latter two studies, is that there are gender differences not only in interoceptive processing as assessed by subjective measures, but that these differences are also present in startle. Future studies should aim to recruit gender-balanced samples to further study gender differences in processing of interoceptive stimuli.

Furthermore, study 4 demonstrated that visceral fear learning to a visceral low intensity sensation is possible. It established that homoreflexive interoceptive conditioning with an esophageal stimulus can effectively induce associative learning. Relevant to future studies is whether learning of associations between low intensity and high intensity visceral stimulation can affect detection, unpleasantness and tolerance thresholds. It would also be relevant to investigate whether homoreflexive visceral fear learning is possible using other types of visceral stimulation, such as gastric or rectal stimulation. And finally, for clinical purposes, it would be very relevant to compare different methods for 'unlearning', i.e. inhibiting learned associations.

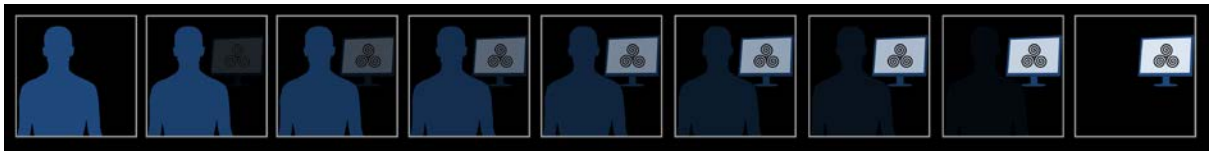

Figure 11. Measure for orientation of attention

This figure is an example of how to possibly measure whether attention is predominantly interoceptive (left hand side), exteroceptive (right hand side), or evenly divided (middle). This measure forms a variation on the more well-known 9-point SAM scales.

General conclusion

Our studies have shown that modulation of startle in interoceptive fear does not always reflect fear or unpleasantness, as would be expected based on studies using visual, auditory and olfactory emotional stimuli. This does not mean fear or unpleasantness are absent: it merely implies that fear and unpleasantness are not the driving factors of startle modulation, especially not in the context of interoceptive stimulation. Currently, startle cannot be considered to be a valid measure of defensive response mobilization during interoceptive stimulation, as there are too many confounding variables with inhibitory and excitatory influences on startle in the context of interoceptive stimulation. Our studies have provided an initial observation that may serve future studies on interoception in predicting when to expect more inhibitory than excitatory influences on startle, and vice versa. These studies are advised to consider either the modulatory role of arousal, that of attention, of fear phase, and/or of the activation of recuperative behavior.

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Appendix

For study 3, the following pictures were selected from the International Affective Picture System (IAPS):

Positive pictures, block A: 1463, 1603, 1620, 1731, 2058, 2209, 2216, 2299, 2311, 2340, 2341, 2345, 2388, 2395, 2398, 2501, 2550, 4532, 4610, 4614, 5001, 5201, 5260, 5480, 5551, 5621, 5623, 5760, 5994, 7230, 7282, 7325, 8370, 8461, 8470, 8499

Positive pictures, block B: 1340, 1710, 1750, 1920, 2165, 2304, 2360, 2387, 2530, 2598, 2660, 4574, 4622, 4626, 4640, 5010, 5700, 5811, 5831, 5833, 5836, 5849, 7280, 7340, 7502, 7580, 8162, 8170, 8185, 8210, 8380, 8420, 8496, 8497, 8502, 8540

Neutral pictures, block A: 1121, 1560, 1670, 1850, 2025, 2038, 2104, 2190, 2191, 2206, 2210, 2214, 2235, 2272, 2305, 2381, 2393, 2396, 2397, 2435, 2480, 2485, 2514, 2579, 2580, 2597, 5395, 5455, 5520, 7002, 7004, 7036, 7140, 7205, 7495, 7640

Neutral pictures, block B: 1675, 1942, 1947, 2102, 2200, 2357, 2372, 2383, 2385, 2445, 2487, 2495, 2499, 2518, 2575, 2593, 2594, 2850, 2870, 2880, 2980, 5471, 5740, 7037, 7041, 7130, 7217, 7491, 7493, 7496, 7504, 7506, 7546, 7550, 8211, 8311

Negative pictures, block A: 1114, 1302, 2095, 2120, 2683, 2691, 2692, 2694, 2703, 2751, 2800, 2811, 3500, 3530, 4621, 5971, 6020, 6190, 6212, 6242, 6312, 6313, 6560, 6838, 6940, 8485, 9001, 9050, 9140, 9270, 9340, 9342, 9409, 9423, 9600, 9900

Negative pictures, block B: 1200, 1932, 2799, 2900, 4635, 5973, 6241, 6250, 6315, 6370, 6550, 6571, 6800, 6821, 6840, 9006, 9041, 9181, 9220, 9230, 9404, 9410, 9417, 9419, 9421, 9424, 9425, 9426, 9429, 9440, 9470, 9520, 9561, 9622, 9800, 9911.

"She knew that this day, this feeling, couldn't last forever.
Everything passed; that was partly why it was so beautiful.

Things would get difficult again. But that was okay too.

The bravery was in moving forward, no matter what."

- *Lauren Oliver*
(in 'Panic')

嬰兒現形圖

此時丹熟更須慈母情嬰兒

氣穴法名無盡藏
歲包於寂寂包空
我問空中誰氏子
他云是你主人翁

衍化生丹
抱候守雌
綿綿若存
念茲在茲

夫婦結之一真
孕蛻蛉之子
傳其精交其
精混其氣和
其神陰陽大
小俱得其真

游龍今已化飛龍
變現神通不可窮
一朝跳出珠光外
渾身直到紫微宮

神水流液
靈源根株
內外無塵
長養聖胎

他日雲飛方見真人朝上帝